The effects of the menstrual cycle and hormonal contraceptives on the central thermoeffector threshold temperatures and width of the interthreshold zone

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Abstract

Basal body temperature (BBT) and thermoeffector thresholds increase following ovulation in many women. This study investigated if solely central thermoregulatory alterations are responsible. Seven females in a non-contraceptive group (NCG) were compared with 5 monophasic contraceptive users (HCG) on separate accounts: pre-ovulation (*Trial 1*; d 2-5) and post-ovulation (*Trial 2*; 4-8 d post-positive ovulation) for NCG, and active phase for HCG (d 2-5, d 18-21). During immersion in 28°C water to the axilla, participants exercised for 20-30 min on an underwater ergometer. After steadily sweating, immersion continued until metabolism increased two-fold due to shivering. Rectal (T_{re}) BBT was not different between trials for neither NCG (1: 37.34±0.16°C; 2: 37.35±0.27°C) nor HCG. At exercise termination, T_{re} forehead sweating cessation increased ($P<0.05$) in trial 2 irrespective of group (1: 37.55±0.39°C; 2: 37.90±0.46°C). T_{re} shivering onset did not increase ($P>0.05$) in trial 2 (1: 36.91±0.50°C; 2: 37.07±0.45°C). The widths of the interthreshold zone increased ($P<0.05$) in trial 2 (1: 0.64±0.22°C; 2: 0.82±0.37°C) due to the increased sweating threshold only. HCG cooled quicker (1: -1.15±0.43°C; 2: -1.00±0.50°C) than NCG participants (1: -0.58±0.22°C; 2: -0.52±0.29°C), and tympanic (T_{ty}) sweat thresholds were significantly ($P<0.05$) decreased (1: 34.76±0.54°C; 2: 35.39±0.61°C) versus NCG (1: 35.57±0.77°C; 2: 35.89±1.04°C). Lastly, T_{re} and T_{ty} thresholds were significantly different ($P<0.05$) for all thresholds within the same trial. In conclusion, BBT is not a reliable indicator of ovulation, only the central thermoregulatory drive for sweating is altered by menstrual phase, contraceptive users have enhanced thermal sensitivities, and T_{ty} opposed to T_{re} provides different measures of core temperature.

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List of Abbreviations Used

17β-estradiol	= OES
Progesterone	= PRO
Oral contraceptive	= OC
Oral contraceptives	= OCs
Follicular phase	= FP
Luteal phase	= LP
Gonadotropin-releasing hormone	= GnRH
Luteinizing hormone	= LH
Follicle-stimulating hormones	= FSH
Synthetic oestrogen	= SO
Synthetic progesterone	= SP
Heat production	= HP
Heat loss	= HL
Basal body temperature	= BBT
Core temperature	= T_c
Core temperature shivering threshold	= $T_{csh'}$
Core temperature sweating threshold	= $T_{csw'}$
Rectal temperature	= T_{re}
Oesophogeal temperature	= T_{es}
Skin temperature	= T_{sk}
Mean skin temperature	= $\bar{T}_{sk}$
Tympanic temperature	= T_{ty}
Interthreshold zone	= ITZ
Rate of oxygen uptake	= $\dot{V}O_2$
Relative rate of oxygen uptake	= $\dot{V}O_2 \cdot kg^{-1}$
Sweat rate	= SR
Perfusion units	= PU
Forearm blood flow	= FBF

Non-contraceptive group	= NCG
Hormonal contraceptive group	= HCG
Analysis of variance	= ANOVA

Chapter 1- Introduction

During thermal stress, sex dictates thermoeffector responses primarily as a function of morphological differences, such as body fat content (Stocks et al., 2004). The notion these differences may also be explained by sex hormones requires clarification. The concentrations of ovarian hormones that fluctuate throughout the female menstrual cycle are accompanied by changes in internal body temperature (Warren & Constantini, 2000). There is general agreement that during the latter half of the cycle, the thermoregulatory set point is higher; consequently, basal body temperature (BBT) and thermoeffector threshold temperatures increase (Hessemer & Bruck, 1985). Findings are inconsistent however, when comparing gains (intensity) of the shivering and sweating responses. Some indicate during the luteal phase (LP), shivering is attenuated (Gonzalez & Blanchard, 1998) and sweating is augmented (Hessemer & Bruck, 1985). Others find shivering and sweating gains are unchanged between phases (Stephenson & Kolka, 1999). Therefore, before the mechanism responsible for sex differences can be defined, how the menstrual cycle alters thermoregulation requires elucidation.

Given that more than 60 million women worldwide rely on the use of orally administered contraceptives, research is warranted to clarify the influences of contraceptive use on temperature regulation (Van De Graaff et al., 1992). According to current knowledge, tolerance time and cardiac strain during heat stress varied throughout the menstrual cycle in females not using contraceptives. However, such phase differences did not exist in a hormonal contraceptive group (HCG) (Tenaglia et al., 1999)—this implies a more uniform response. On the other hand, similar to non-hormonal contraceptive users, BBT and the thermoeffector threshold temperatures remain increased during the active hormone phase (Charkoudian & Johnson, 1997). Results also exhibit changes in shivering, but not in sweating gain (Grucza, et al., 1993).

Due to female-specific changes, human thermoregulation has been investigated primarily in males. Even more, the little research examining females is isolated to the follicular phase (FP) when sex hormones are lowest in concentration. Drinkwater et al. (2000) further notes that research has been

influenced by erroneous methods. Errors include inaccurate determination of the menstrual phase, variations in basal hormones from chronic training or former exercise bouts, limited sample sizes, dissimilar fitness status, and assorted contraceptive prescriptions.

Throughout these early investigations, participants were exposed to various thermal loads over the menstrual cycle course, and thermal responses were monitored both peripherally and centrally. However, the thermal influence of sex hormones -oestrogens and progestogens- are believed to be solely central (Carpenter & Nunneley, 1988). A model by Mekjavic et al. (1991) was created to clamp mean skin temperature ($\overline{T}_{sk}$) involving exercise and subsequent passive cooling while immersed throughout at a constant water temperature. Thus peripheral thermoafferents are removed, and central sweating and shivering temperature thresholds can be identified (Mekjavic et al., 1991). This same model was used to determine morphological sex differences in response to thermal stress; however females were only tested during one phase of the cycle (Anderson et al., 1995). Therefore, it is valuable to investigate females using the same heating and cooling immersion protocol but taking into account the entire menstrual cycle.

It is important to broaden the understanding of thermal effects from the menstrual cycle, when approximately 8,500,000 women in Canada are affected by cyclical hormone fluctuations (Statistics Canada, 2006). Furthermore, contraceptive use is highly popular among females due to successful anti-fertility rates, and associated health benefits (Drinkwater et al., 2000). In addition, female participation in manual labour and athletic events has grown dramatically in recent years (Drinkwater et al., 2000). The percentage of females competing in the 1904 St. Louis Olympic Games was 0.9% (1078 participants), increasing to 41% (4329 participants) in the 2004 Greece Summer games (International Olympic Committee, 2008). Industrial work, and athletic competition occur in variable conditions that challenge women to take on physically demanding activities in extreme heat and cold (Drinkwater et al., 2000). The hormonal shifts may predispose females to heat illness or cold injury, and may require modified behavioural thermal strategy to maintain optimal physiological function.

By conducting this study, a greater understanding will be gained about oestrogens and progestogens, and their involvement with the hypothalamus to regulate temperature. Results will provide

insight on the thermoregulatory differences between sexes, and confirm if thermophysiology is dependent on menstrual cycle status. For this investigation, participants will be tested in both menstrual cycle phases. Involvement will include normally ovulating females, and a control group prescribed a monophasic contraceptive, tested only in the active phase of hormone delivery. By stabilizing $\overline{T}_{sk}$, and utilizing a "control" group with constant hormonal milieu, results will indicate how fluctuating sex hormones affect central control of vasomotor, sweating, and shivering thresholds, and their gains during both hot and cold temperature stress.

Chapter 2- Review of Literature

Historically, research has focused on thermoregulatory responses in young, adult males. However, the importance of sex-dependent research is recognized more widely as it reflects the increase of female participation in activities that are physically demanding, such as athletics (Drinkwater et al., 2000), and industry (Vatter, 1994). When the influence of sex on autonomic thermoregulation is examined, data is variable—possibly a resultant of sex-specific hormones. It remains unclear how circulating oestrogens and progestogens affect the central control of temperature regulation throughout the menstrual cycle.

For greater understanding, basic information on sex hormones, the physiological events of the menstrual cycle, and the pharmacology of hormonal contraceptives (HC) are discussed. Subsequent sections include temperature regulation during thermal stress, and review of the literature quantifying thermoregulatory response in females as a consequence of endogenous and exogenous sex hormones. In the final section, gaps within the literature are elaborated on. A primary focus of this review is to note current trends and conflicts within previous literature. It will not prove or disprove the manner in which humans thermoregulate, but rather select one probable model and examine how female hormones influence the activation and sensitivity of thermal response with respect to that model. In addition, this review will not discuss physiological alterations occurring throughout the menstrual cycle not directly associated with temperature regulation. Fluid, metabolic, and cardiovascular alterations have also been noted between phases but are detailed by others (Drinkwater et al., 2000).

1) Female Sex Hormones

i) Secretion

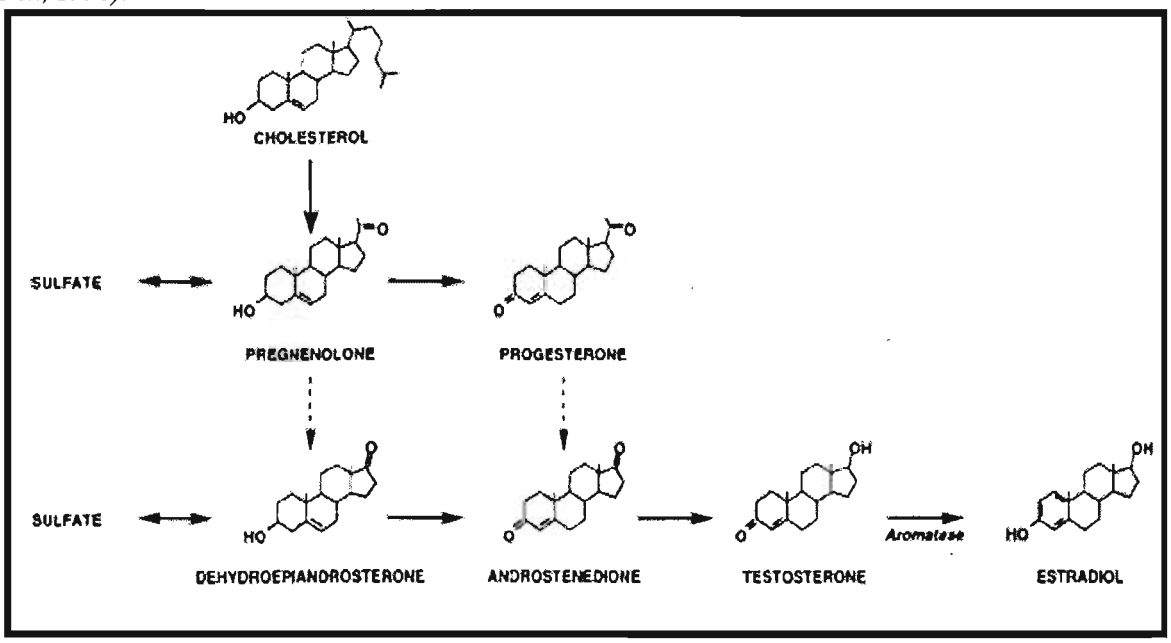
Female endocrinology is well understood, and clearly described by Fox (2000). The hypothalamus secretes gonadotropin releasing hormone (GnRH) following puberty. GnRH promotes the anterior

pituitary gland to secrete the gonadotropic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH are termed as such because their secretions cause the gonads (ovaries) to form egg cells that secrete oestrogens and progestogens. Oestrogens and progestogens are the dominant female sex hormones that maintain ovarian function (Fox, 1990). There are multiple oestrogens and progestogens, but 17β-oestradiol (OES) and progesterone (PRO) circulate in the greatest concentrations, and thus cause the majority of the reproductive effects (Johnson, 2008). Therefore, OES and PRO will represent all oestrogens and progestogens in this review.

ii) Synthesis

There are numerous hormones secreted by the endocrine system, yet only the steroid hormones are pertinent to this review. OES and PRO are two steroids derived from cholesterol precursors (Griffin & Ojeda, 2000). The functional chemical groups attached to the cholesterol hydrocarbon backbone will distinguish one hormone from the other (Fox, 1990). PRO is synthesized via direct conversion (aromatization) from a derivative of cholesterol (Fox, 1990). OES synthesis is more complex, requiring PRO aromatization and subsequent aromatization of associated sex hormones, androstenedione and testosterone (Fox, 2000). Simplified below (*Figure 2.1.*) is a schematic of OES and PRO synthesis.

Figure 2.1. Biochemical synthesis of oestradiol and progesterone from the cholesterol precursor (from Fox, 1990).



iii) Transport

Unlike nervous system transmission, communication from sex hormones may continue for days (Saladin, 2001). OES and PRO are released into the bloodstream and transported to their target cells loosely bound to plasma-binding proteins. Following transport, OES and PRO attach to receptor sites on the target cells and form hormone receptor-complexes (Griffin & Ojeda, 2000). The complexes are contained within the nucleus, but may also be present on the membrane or in other organelles. Once the high affinity OES and PRO complexes are formed, the receptors' structures are altered and activated to synthesize new proteins (Griffin & Ojeda, 2000). These proteins then carry out the typical hormonal responses on the target cells (Saladin, 2001). Dependent on the hormone and cell interactions, these responses occur immediately at the membrane of the target tissue, or are fed forward to release other hormones eventually altering the target cells (Griffin & Ojeda, 2000).

2) The Menstrual Cycle

i) Review

It is further important to understand the function of the female sex hormones during the course of the menstrual cycle. The cooperative secretion, synthesis, and transport of the hormones stimulate the events that comprise the menstrual cycle phases (Van De Graaff et al., 1992). Van De Graaff et al. (1992) described the cycle as it spans over the approximate 28-day time course. In brief, the menstrual phase occurs on day one and typically lasts through day five. The follicular phase (FP) is to follow—days 5 to 14—when an egg cell grows, transforms into a follicle, and progressively secretes OES. When LH and OES reach critical levels on approximately day 14, the follicle is ovulated and released. During the luteal phase (LP)—days 15 to 28—primarily PRO is secreted from the follicle until cell death. When PRO and OES concentrations decrease, the beginning of the cycle is reached again. The following table (*Table 2.1.*) summarizes the principal functions of the sex hormones, while the figures (*Figures 2.2. and 2.3.)* depict the normal concentration fluctuations throughout the cycle.

Table 2.1. Functions of the female sex hormones (Johnson 2008) and their hypothesized thermal physiological effects.

Hormone	Functions	Thermal physiological effects
GnRH	• Secreted from the hypothalamus • Initiates secretion of LH and FSH	
LH	• Secreted from the anterior pituitary gland • Triggers ovulation • Converts the follicle into the corpus luteum	
FSH	• Secreted from the anterior pituitary gland • Initiates follicular growth	
OES	• Secreted primarily from the follicles in the ovaries • Promotes female secondary sex characteristics • Causes the LH surge to trigger ovulation • Thickens the endometrium • Inhibits further LH and FSH secretion	• Decreases basal body temperature (Carpenter & Nunneley, 1988) • Decreases core temperature during exercise (Carpenter & Nunneley, 1988) • Decreases sweating threshold (Stephenson & Kolka, 1999)
PRO	• Secreted from the follicles in the ovaries • Develops the fetus • Increases water and sodium excretion from the kidneys • Inhibits further LH and FSH secretion	• Increases basal body temperature (Hessemer & Bruck, 1985) • Decreases thermal conductance in a neutral environment (Frascarolo et al., 1990) • Increases sweating and vasodilator thresholds (Kolka & Stephenson, 1989) • Increases shivering thresholds (Hessemer & Bruck, 1985)

Figure 2.2. Fluctuations of the gonadotropic sex hormones throughout the menstrual cycle (modified from Marieb, 1988).

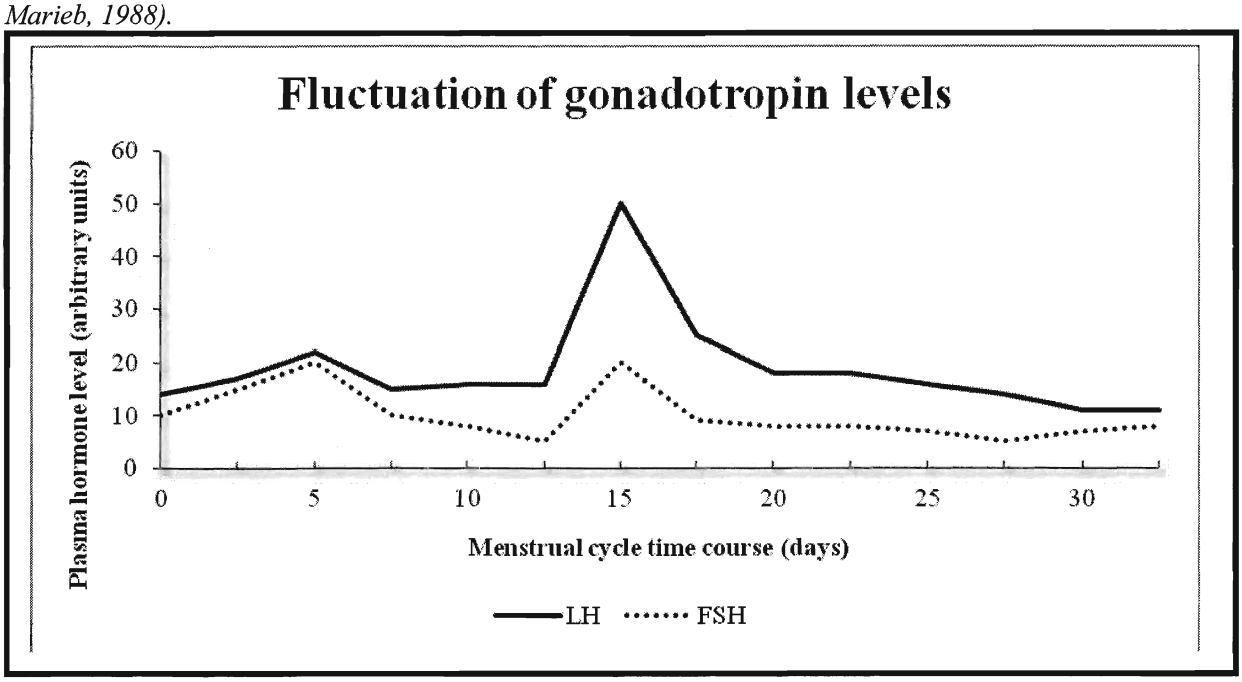
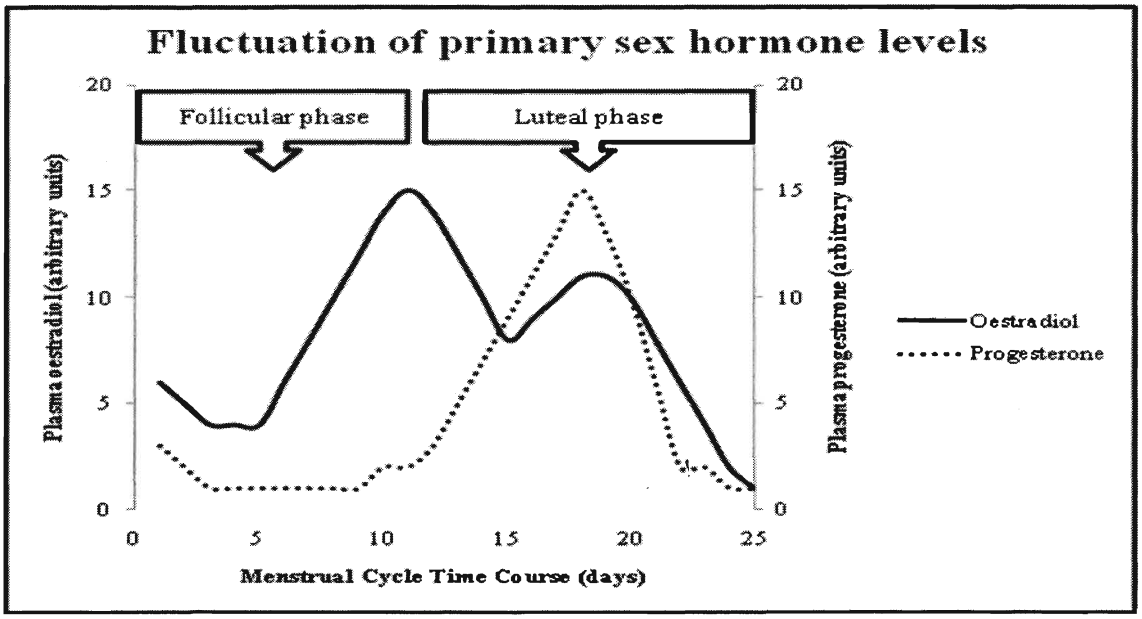


Figure 2.3. Fluctuations of the ovarian sex hormones throughout the menstrual cycle (modified from Marieb, 1988).



3) Hormonal Contraceptives

i) Review

There are multiple forms of HC available. Their prevalence has increased, with approximately 60 million worldwide prescribed on oral ingested contraceptives alone (Van De Graaff et al., 1992). Most contraceptives are combination in nature, and consist of synthetic oestrogens (SO) and synthetic progestogens (SP). With administration on "day one," contraception is achieved as plasma SP immediately increases. Greater SP content deters FSH and LH secretion, disallows follicle maturation, thus OES secretion. Positive feedback from OES does not occur, prevents the surge in LH, and inhibits ovulation (Van De Graaff et al., 1992). Depending on the type of contraceptive, SO and SP concentrations can vary or remain the same. Regardless of the type, one package provides three weeks of exogenous hormonal dose, called the active phase. In the fourth week, the contraceptive is no longer administered, called the withdrawal phase. During withdrawal, SO and SP concentrations quickly decrease, and allow for menstruation (Fox 2000). The table below (*Table 2.2.*) summarizes the three different forms of combination contraceptives offered.

Table 2.2. Combination contraceptive formulae.

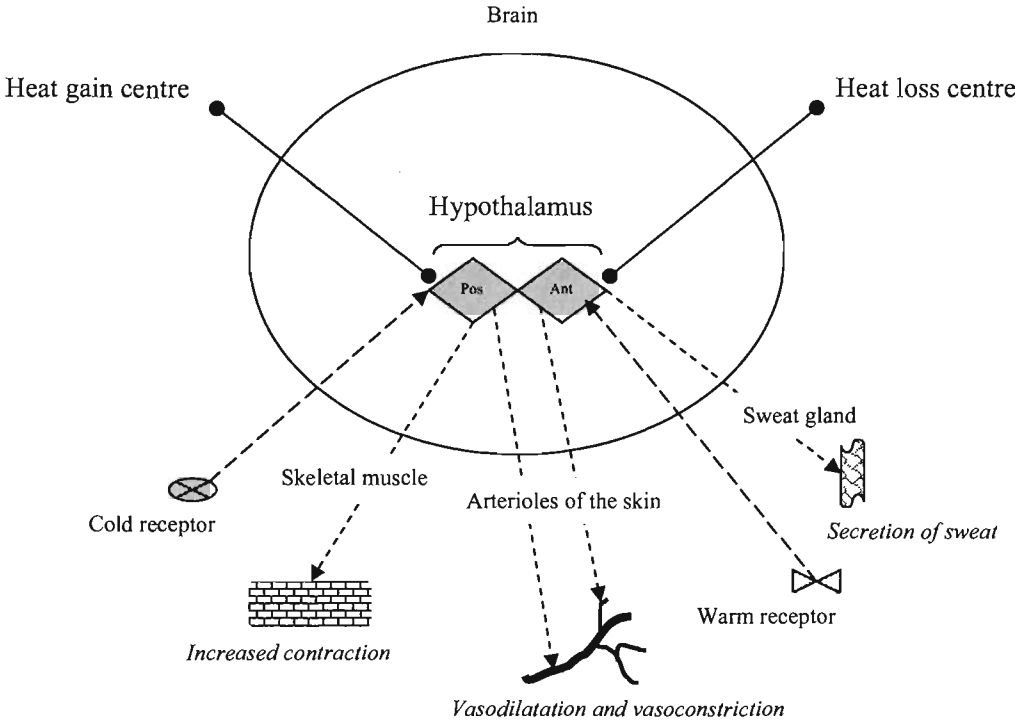
Combination (SO and SP)	**21 active doses, 7 placebo or inactive doses**
Monophasic	21 d steady hormone dose
Biphasic	Hormone dose is changed once throughout the active formulation; reduced total hormone dose
Triphasic	Varying hormone content every 7 d; best mimics endogenous hormone shifts; least amount of total hormone dose

4) Temperature Regulation

i) Review

Having discussed female specific endocrinology, it is important to clarify particular facets of autonomic thermoregulation. During acute exposure to extreme cold or hot environments, physiological strain increases. The thermoregulatory and cardiac systems work synchronously to limit core temperature (T_c) change by regulating heat storage. For optimal function and health, T_c maintains relatively constant—approximately 37°C—rendering humans as homoeothermic (Folk, 1974). The hypothalamus contains cold and warm sensitive neurons, located in different regions of the pre-optic anterior region (see *Figure 2.4.*) (Reilly & Waterhouse, 2005). Temperature insensitive neurons are also found in this region (Silva & Boulant, 1986). The temperature sensitive neurons receive thermal afferents from peripheral and central thermoreceptors. Those in the periphery are located variably throughout the skin-surfaces, and mucous membranes. The spinal cord, brain and hypothalamus contain the temperature sensitive neurons specific to the core (IUPS, 2001). After the hypothalamus integrates the thermal information from both receptor-types, effector organs are stimulated to initiate heat production or heat loss (Marieb, 1988). During exposure to the cold, metabolic heat is generated and stored in the body. Conversely, excess metabolic heat that is produced in the heat is dissipated. The amount of heat-energy transferred is predominantly dependent on the duration and severity of the environment (Marieb, 1998), while the magnitude and direction determine the extent of T_c change.

Figure 2. 4. Nervous system control of the effector organs associated with cold and hot temperature physiology (from Reilly & Waterhouse, 2005).



ii) Cold Stress Physiology

Upon activation of the posterior hypothalamus, two effector mechanisms are initiated, cutaneous vasoconstriction and shivering thermogenesis. During vasoconstriction, tissue insulation increases to reduce convective heat loss up to one-third (Folk, 1974). When the cold stress is substantial, skeletal muscles contract to produce heat, further conserved with vasoconstriction (Stocks et al., 2004). These involuntary-asynchronous-contractions can elevate metabolic heat production four-to-six fold (Folk, 1974).

iii) Heat Stress Physiology

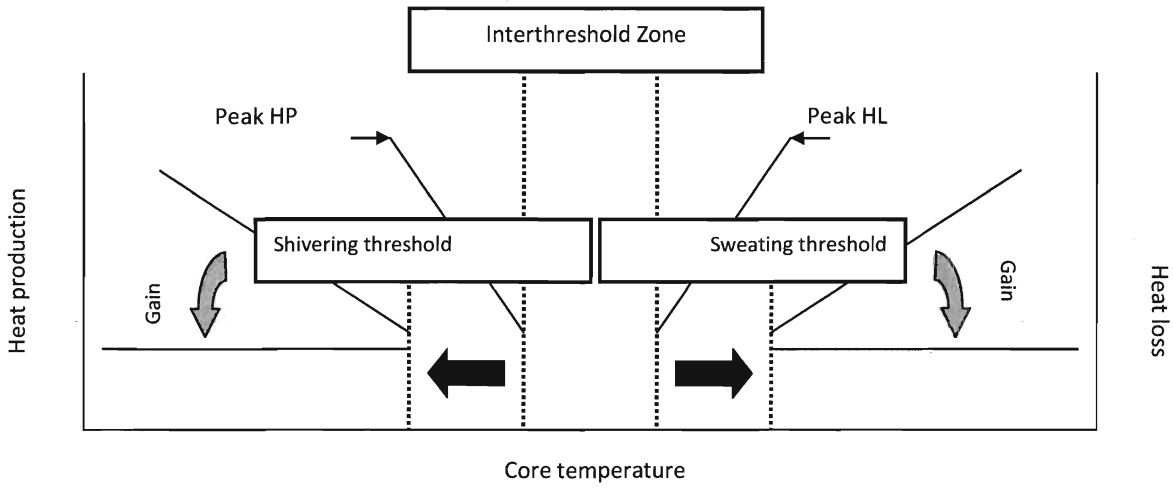
The warm-sensitive-anterior-hypothalamus processes thermal afferents signalling that blood, T_c, and skin temperature (T_{sk}) are exceedingly warm. These impulses activate two effector responses,

cutaneous vasodilatation and sweat gland secretion. As blood vessel diameter increases, blood shunts to the periphery, and central blood flow reduces (Folk, 1974). Vasodilatation dissipates the heat in the blood by increasing dry heat exchange to the environment (Marieb, 1988). If the heat stress is great enough, the eccrine sweat glands are triggered to release biological fluid. Sweating removes heat through the phase change and evaporation of the water off the skin's surface (IUPS, Thermal Commission, 2001).

iv) Interthreshold Zone

The responses to cold and heat stress are well understood, though it is debated precisely how hypothalamic temperature is perceived and thermoeffector responses initiated (Flouris, 2008). There are multiple models proposing the mechanisms of neuronal activation and integration of peripheral and central afferents. Some favour a model in which effectors are activated when an internal set point temperature deviates from a consistent value (Hammel, 1968); this "set point" theory suggests T_c is continuously compared to a reference temperature, and fires an error signal when changed by a particular value (Cabanac & Massonnet, 1977). The error signals diminish eventually when vasomotion, shivering or sweating are activated (Mekjavic & Eiken, 2006). In turn, a large deviation from the reference temperature is avoided (Hammel et al., 1968). Others conclude that sweating and shivering occur at particular thresholds, and are absent through a range of internal temperatures (Mekjavic, 1991). In this "interthreshold zone" (ITZ) theory, neither shivering nor sweating occur as long as T_c is within temperature limits (Mekjavic, 1991). Kakitsuba et al. (2007) demonstrated shivering and sweating are also examined along an abscissa of peripheral temperatures to indicate the presence of both peripheral and central ITZs. Whether the thresholds are identified using T_c or T_{sk}, only vasomotor activity occurs within the ITZ, and precedes the onset of sweating and shivering as depicted in the figure below (*Figure 2.5.*). However, there is variance in thermal thresholds and intensities (gains) due to inter-individual and non-thermal factors (Kakitsuba et al., 2007) including exercise, blood glucose, hydration state, sleep, motion sickness, and fever (as referenced by Mekjavic & Eiken, 2006).

Fig. 2.5. Core temperature interthreshold zone (from Mekjavic & Eiken, 2006). Note the variability of thresholds and gains for each effector response as represented by the arrows from individual and non-thermal factors.



v) Activation and Intensity of Thermoregulatory Responses

When identifying the T_c that corresponds with vasomotor, thermogenic (shivering) or sudomotor (sweating) onsets, the terms *thermoeffector threshold* and *thermoeffector threshold temperature* are used. The IUPS Thermal Commission (2001) defined the threshold as: "The level of activity of a potential thermoeffector that is transgressed when it becomes actively involved in temperature regulation," and the threshold temperature as: "The level of a specified body temperature...which in one direction, either upward or downward, will activate a certain thermoeffector" (IUPS Thermal Commission, 2001). Experimentally there are many ways to quantify vasomotor thresholds, such as forearm blood flow (Brengelmann & Savage, 1997), local T_{sk} (Daanen, 2003), venous occlusion plethysmography (Hessemer & Bruck, 1985) and laser-Doppler flowmetry (Wilson et al., 2007). Studies quantifying shivering thresholds utilized electromyography (Bittel et al., 1988) or rate of oxygen uptake ($\dot{V}O_2$) (Anderson et al., 1995). Shivering onset occurs when $\dot{V}O_2$ doubles the resting rate (Mekjavic et al., 1991), and reflects the contractile forces of large muscles. Although there are multiple methods, to determine sweating onset, ventilated sweat capsules can be utilized (Carpenter & Nunneley, 1988).

Another important thermal characteristic is gain. One common method of calculating gain is through portraying the response's activity as a function of T_c and calculating the slope of the linear plot created; a steeper slope indicates greater response intensity in a given thermal load, as shown by the grey arrows in *Figure 5.* and the angle of the diagonal line with the horizontal (Kolka & Stephenson, 1989).

Both the threshold and gain of a thermophysiological response affect the rate of change in T_c. During cold stress, quicker vasoconstriction onset will enhance heat preservation, maintain thermal balance without maximal shivering, and delay time to hypothermia (Haman, 2006). Conversely, quicker vasodilatation onset enhances heat loss, while earlier and greater sweat output attenuates risk of hyperthermia (Armstrong, 2000).

At present, there are substantially more data on male thermal response. As a result, further research is required on female specific thermal thresholds and gains. Provided that T_c fluctuates due to endocrinology, thermoregulation is more variable, and may increase risk of hypo- or hyperthermia in extreme conditions.

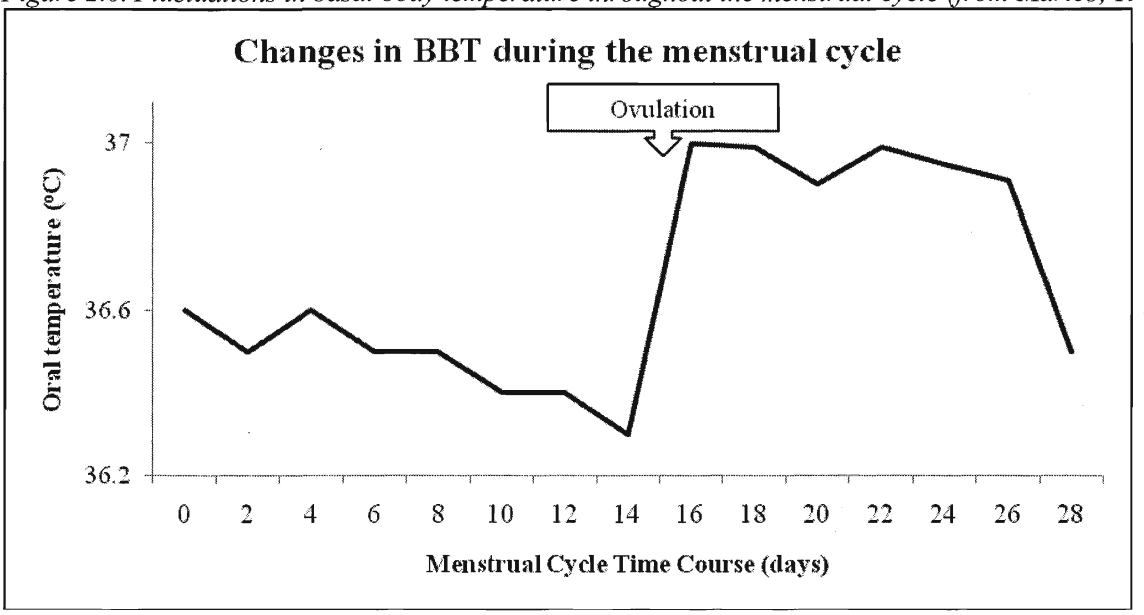
5) Menstrual Cycle and Thermoregulation

i) Review

In previous findings, sex affected rates of relative heat storage (McLellan, 1998), sweating dynamics (Bittel & Henane, 1975), and thermogenesis (McArdle et al., 1984), as a result of different body morphology and composition: likely sex hormones have an influence as well. Literature comparing sexes conducts tests during the FP only when concentrations of OES and PRO are low, and the hormonal milieu less influential (Kaciuba-Uscilko & Grucza, 2001). However, studies that examine females across the entire menstrual cycle indicate thermoregulation changes as hormones fluctuate. It is well documented that T_c increases 0.3°-0.7°C following ovulation, and is sustained during the LP when PRO content is greatest (Gonzalez & Blanchard, 1998; Hessemer & Bruck, 1985; Horvath & Drinkwater, 1982). Conversely, there is a rapid decrease in T_c when OES content peaks prior to ovulation (Stephenson &

Kolka, 1999). The figure below (*Figure 2.6.*) demonstrates these shifts in basal body temperature (BBT) pre- and post-ovulation in normally menstruating females. Since T_c fluctuations occur, sex-specific thermoregulation remains ambiguous.

Figure 2.6. Fluctuations in basal body temperature throughout the menstrual cycle (from Marieb, 1988).



Although there are numerous hypotheses, a probable mechanism of BBT shifts is the hormones altering firing rates of the temperature-sensitive neurons (Nakayama et al., 1975; Silva & Boulant, 1986). Nakayama et al. (1975; action of PRO on preoptic thermosensitive neurons) administered PRO to rats, and noted the firing rates of warm-sensitive neurons decreased, while those of cold sensitivity increased; heat loss responses dampened, heat production was activated, and BBT increased. Further, Silva & Boulant (1986) found with OES administration to hypothalamic tissue slices, activity of the warm-sensitive neurons increased, whereas the cold-sensitive neurons were inhibited or unaffected. In this experiment, heat loss was promoted, and therefore, decreased BBT.

Similar shifts in BBT have been documented in humans, as well as alterations to thermoeffector thresholds and gains. It is unsurprising that during the LP, heat loss and heat production are activated at higher internal temperatures when BBT is increased (Hessemer & Bruck, 1985). The following section

will review the data, and compare thermoregulatory responses between the FP and LP during cold, hot and/or exercising stresses.

ii) Cold Stress Phase Differences

Greater PRO content stimulates metabolic heat production and raises BBT in a thermoneutral condition. Therefore, when exposed to cold stress, T_c thresholds for further heat production increase to allow for vasoconstriction and shivering activation at higher absolute T_c (Hessemer & Bruck, 1985). Given that BBT is shifted upwards, heat production at a higher T_c accommodates this change. Furthermore, shivering gain is reduced during the LP; presumably, there is less need for intense shivering at higher temperatures (Gonzalez & Blanchard, 1998). In agreement, Hessemer & Bruck (1985) found that post-ovulation, the rate of heat debt is decreased during passive cold stress. Interestingly, heat production is activated following similar relative decreases in T_c regardless of menstrual phase, though the intensity of the vasoconstriction and shivering responses will be dulled post-ovulation. Taken together, cold tolerance may improve during the LP with an increase in, and maintenance of, higher BBT. To highlight specific experiments subjecting females to cold throughout both menstrual phases, refer to *Table 2.3.* below.

Table 2.3. Summary of the literature- the menstrual cycle and cold responses.

Reference	**Subjects**	**Thermal load**	**Significant results**
Kenshalo (1966)	3 untrained females	• Detection of peripheral cold sensation thresholds following manipulation of initial T_{sk} (30° - 40°C) • Used copper/bismuth plates placed over the forearms for thermal stimuli • Daily testing throughout three consecutive menstrual cycles • Menstruation vs. FP vs. LP	<u>All results during LP and when T_{sk} adapted to higher temperatures only</u> • **Increased cold sensitivity** • Increased vasodilatation
Hessemer & Bruck (1985)	10 females: 9 untrained, 1 trained	• Ramp cooling from 32° to 12°C until metabolic rate increases 100% • Subjects passive	<u>All results during LP</u> *Pre-exposure:* • Increased T_c • Increased T_{sk}

		• FP (4-7 d post-menstruation) vs. LP (4-8 d post-ovulation)	• Increased basal metabolic rate • Increased arm blood flow • Increased heart rate *Cold exposure:* • **Shivering onset threshold increased** • Decreased respiratory exchange ratio while shivering • **Decreased electrical muscle activity while shivering**
Gonzalez & Blanchard (1998)	6 trained females	• 80 min ramp cooling from 20° to -5°C • Donned 2 ensembles: *A)* less thermal resistance, *B)* more thermal resistance • FP (d 2-6) vs. LP (d 19-23)	<u>All results during LP</u> *Pre-exposure for both ensembles:* • Increased T_c *For A ensemble only:* • Decreased end T_c • **Decreased rate of heat debt** • Increased mean heat flux due to decreased vasoconstriction *For both ensembles:* • Decreased shivering gain • No phase differences in shivering threshold
Klentrou et al. (2004)	7 pre-menarcheal, 6 menarcheal adolescent females	• 40 min cycling at 30% maximal oxygen uptake in 5°C • FP (d 1-5) vs. LP (d 19-22)	• Trends for greater thermal comfort and less thermal sensitivity during FP • **No phase differences in shivering response or T_c**

As indicated above, females may be better protected during the LP in the cold. Tolerance is enhanced post-ovulation: the rate of heat debt decreases (Gonzalez & Blanchard, 1998), as the shivering threshold (Hessemer & Bruck, 1985), and peripheral cold sensitivity increase (Kenshalo et al., 1966).

Unfortunately, these studies cannot confirm the mechanism causing such phase differences, and report various hypotheses as a consequence. For instance, Gonzalez and Blanchard (1998) suggested decreased shivering intensity due to competitive inhibition between the cold afferents and warming effect of PRO in the hypothalamus. Meanwhile, the lack of cold response differences in the menarcheal group from the study by Klentrou et al. (2004) does not provide mechanistic insight, except for an understanding that phase differences rely on gynaecological age. Most are in agreement, however, that the sex hormones

are centrally acting, and thus affect the entire thermoregulatory system and multiple organs. This central hypothesis is supported by data illustrating the thresholds for sweating and shivering are altered in the same direction, and to a similar magnitude (Hessemer & Bruck, 1985). Furthermore, the adjustments to BBT are in opposing directions, but comparably concentration dependent, pre- and post-ovulation (Marrone et al., 1976). These parallel alterations would not occur if the hormonal effects were solely peripheral in nature as hypothesized by Kenshalo (1966). In this study, phase shifts were noted in vasomotor activity and sensitivity only, and not BBT (Kenshalo et al., 1966). The protocol used, however, was highly influenced by the periphery since cooling was applied to only one surface region of the body.

It is evident from the aforementioned studies, that a precise mechanism for phase alterations is unknown. Whether changes are centrally or locally driven remains unclear as both cutaneous and whole body responses differed. Protocols involving transient cooling, local skin cooling, and exercise are confounded by changing T_{sk} and peripheral thermoafferents. Better control of thermal input from the periphery is required to elucidate whether PRO and OES affect only the hypothalamus, the periphery, or their integration.

iii) Heat Stress Phase Differences

Literature supports that the increase in BBT during the LP remains elevated during exercise or heat exposure (Pivarnik et al., 1992). Further, activation of heat loss—vasodilatation and sweat—is delayed to greater absolute T_c values when inclined to regulate at higher temperatures (Hessemer & Bruck, 1985; Kolka & Stephenson, 1989; Stephenson & Kolka, 1999). It is logical that thermoeffector thresholds shift. Otherwise, convective and evaporative heat loss would occur when normothermic, and profuse sweating would occur during heating or exercise. In addition, some found sweat gain increases to oppose a higher T_c (Hessemer & Bruck, 1985). However this finding is not consistent, and is demonstrated in *Table 2.4.* following a comparison of protocols that increase T_c throughout both phases of the menstrual cycle.

Table 2 4. Summary of the literature- the menstrual cycle and hot responses.

Reference	Subjects	Thermal load	Significant results
Horvath & Drinkwater (1982)	4 untrained females	• 25 min walking at 30% maximal oxygen uptake in 28°C, 35°C, and 48°C • Menstruation vs. ovulation vs. LP	<u>All results during LP</u> *Pre-exposure:* • Increased resting T_c • Increased resting $\dot{V}_{O2}$ *28°C only:* • Decreased forearm blood flow • Decreased T_{sk} *35°C only:* • Decreased stroke volume • Increased T_c when t= 5 min, 25 min 48°C only: • **Increased evaporative heat loss** • Greater relative decrease in plasma volume
Hessemer & Bruck (1985)	10 females: 9 untrained, 1 trained	• Ramp heating from 18° to 59°C until 12-15 min post-sweat onset • Subjects passive • FP (4-7 d post-menstruation) vs. LP (4-8 d post-ovulation)	<u>All results during LP</u> *Pre-exposure:* • Increased T_c • Increased T_{sk} • Increased basal metabolic rate • Increased arm blood flow • Increased heart rate *Heat exposure:* • **Increased chest sweat threshold** • Increased vasodilatation threshold • Increased cutaneous heat conductivity (heat flux) • Increased rate of change in cutaneous heat conductivity • Increased T_c • Increased T_{sk} • Increased chest sweat rate • **Increased sweating gain** • Increased heat conductivity gain
Kolka & Stephenson (1989)	7 untrained females	• 160 min in 50.4°C • Subjects passive • 9 min cycling at 80% maximal oxygen uptake in 50.4°C • 35 min cycling at 85% maximal oxygen uptake in	<u>All results during LP</u> • **Increased sweating threshold** • Increased T_{sk} • No phase differences in sweat gain • Greater norepinephrine

		35°C • FP (d 4-7) vs. LP (d 19-22)	content • Decreased subjective tolerance
Stephenson & Kolka (1999)	6 females: 4 trained soldiers, 2 civilians	• Walking to volitional exhaustion in 30°C • Early FP (d 2-6) vs. late FP (d 9-12)	<u>All results during late FP</u> • Decreased resting T_c • Decreased sweating threshold • **No phase differences in sweat gain**

In brief, females may be at a disadvantage in the heat during the LP. Vasodilatation and sweating are delayed, concurrent with elevated BBTs (Hessemer & Bruck, 1985; Kolka & Stephenson, 1989). Physiological strain is exacerbated as norepinephrine concentration (Kolka & Stephenson, 1989), heart rate (Hessemer & Bruck, 1985), $\dot{V}O_2$, and fluid losses (Horvath & Drinkwater, 1982) increased more during exercise; T_c and T_{sk} are higher, and reduce the thermal gradient to lower heat dissipation capacity (Kolka & Stephenson, 1989; Hessemer & Bruck, 1985). There is debate whether or not sweat gain increases, as only some found enhanced sweat sensitivity (Hessemer & Bruck, 1985). As a result, it is unclear whether central sensitivity or sweat gland output is altered when peripheral and central thermoregulation is not separated. Further, phase differences may be masked when exercise and heat stress are combined, versus heating passively (Horvath & Drinkwater, 1982).

The experiments summarized above imply increased PRO concentration is responsible for vasomotor and sudomotor threshold delays. As previously described, PRO is hypothesized to increase cold-sensitive neuronal activation, and regulates the core at elevated temperatures (Nakayama et al., 1975): heat loss thresholds are adjusted accordingly. Vasodilatation and sweating thresholds are activated following similar relative T_c increases as the FP, but occur at a higher absolute T_c during the LP. Ultimately, the precise mechanism whereby increased PRO triggers heat loss phase differences is elusive. A protocol is required to segregate peripheral and central afferents to determine how the thermoeffector thresholds and gains are affected by fluctuating sex hormones.

6) Hormonal Contraceptives and Thermoregulation

i) Review

Given the prevalent use of HC, thermoregulation in conjunction with exogenous hormone use warrants investigation. Current knowledge shows that despite consistent hormonal doses, BBT and thermoeffector thresholds significantly increase in the latter course of hormone delivery (Charkoudian & Johnson, 1999; Grucza et al., 1993). These shifts are not influenced by HC but must result from an inevitable influence of the menstrual cycle, provided that the same shifts occur in females not utilizing HC (Grucza et al., 1997). Data investigating thermoregulatory gains however, are more controversial. Some showed HC minimized differences during exercise stress (Grucza et al., 1993), while others found temperature regulation differences only in HC users (Armstrong et al., 2005). In other words, HC may alter the intensities of thermal responses due to a more subtle impact from the menstrual cycle, and contribute to conflicting findings (Grucza et al., 1997).

ii) Cold Stress Phase Differences

As BBT increases near the end of the active phase, shivering and vasoconstriction T_c thresholds shift upwards (Charkoudian & Johnson, 1999; Grucza et al., 1997). Elevated absolute onset temperatures cause T_c to regulate around a higher value throughout cooling. However, shivering gain data is equivocal. Some indicated intensity significantly increased during the quasi-FP (Grucza et al., 1997), while others found it is unaltered between low and high hormone phases (Charkoudian & Johnson, 1999). The former study compared females during the high dosage only, while the latter compared females during both placebo and high hormone dosages. Therefore, hormonal status was different between studies. To further compare the documentation, below is *Table 2.5.* highlighting some protocols, and pertinent results of females using HC exposed to cold.

Table 2.5. Summary of the literature- hormonal contraceptives and cold response.

Reference	Subjects	Thermal load	Significant results
Grucza et al. (1997)	• 7 untrained females • All monophasic HC prescribed	• 30 min in 3.4°C • Subjects passive • Quasi-FP (d 9-10) vs. quasi-LP (d 22-23)	<u>All results during quasi-LP</u> *Pre-exposure:* • Increased BBT *Cold exposure:* • Increased T_c • **Increased shivering threshold** • Increased time to shiver onset • **Decreased shivering gain** • Decreased heart rate • Decreased respiratory rate
Charkoudian & Johnson (1999)	• 8 untrained females • All monophasic HC prescribed	• Ramp cooling of T_{sk} from 36°C at a rate of $0.2°C \cdot min^{-1}$ for 12-15 min • Water perfused suit • Subjects passive • Low hormone phase vs. high hormone phase (precise days undefined)	<u>All results during high hormone phase</u> *Pre exposure:* • Increased BBT *Cold exposure:* • Increased T_c • **Increased cutaneous vascular conductance threshold (vasoconstriction threshold)** • **No phase differences in cutaneous vascular conductance slope**

These studies confirm vasoconstrictor and shivering onsets occur at elevated thresholds, and support the endogenous effect of the menstrual cycle overrides HC influences on BBT and activation thresholds. However, whether cold sensitivity is dampened or unaltered between phases remain uncertain. All subjects were prescribed a monophasic HC, yet testing took place during different HC doses, likely causing disparate findings. Had the females been tested during the inactive phase in the study by Grucza et al. (1997), a clearer understanding of effector gains would result, and significant differences may have disappeared. Furthermore, either study did not compare with non-HC users that could provide further insight.

Interestingly, Grucza et al. (1993) found sweating gain was indifferent between the quasi-FP and quasi-LP. Alterations in only shiver gain suggest HC affect heat loss and production through different

mechanisms. On the other hand, Charkoudian & Johnson (1997) found vascular response is equally altered during heat and cold stress. These findings would support SO and SP act in the same manner, and that the mechanism is centrally driven. Although conflicting findings may be attributable to methodology, data is limited, and further investigation is required to better understand the influence of HC on cold response sensitivity.

iii) Heat Stress Phase Differences

Although females that utilize HC exhibit an upwards shift in the temperature set point mid-hormone delivery, physiological stress in the heat is suppressed. In normally ovulating females, cardiovascular strain increases during heat exposure post-ovulation. In contrast, females with high SO and SP content portrayed decreases in heart and sweat rates for a given T_c (Charkoudian & Johnson, 1997). Furthermore, HC use did not alter sweating intensity, (Grucza et al., 1993), vasodilator gains (Charkoudian & Johnson, 1997), and exercise tolerance time across active and withdrawal phases (Tenaglia et al., 1999). Taken together, during transient increases in T_c, thermoregulation is more uniform between menstrual phase, and physiological strain is minimized with HC use. The data are summarized in *Table 2.6.* below, that investigate the influence of HC during exposure to heat throughout the menstrual cycle.

Table 2.6. Summary of the literature- hormonal contraceptives and hot response.

Reference	**Subjects**	**Thermal load**	**Significant results**
Grucza et al. (1993)	• 20 untrained females • 8 triphasic HC prescribed, 2 monophasic HC prescribed • 10 non-HC users	• 45 min cycling at 50% maximal oxygen uptake in 24°C • Quasi-FP vs. quasi-LP (precise days undefined) • FP (d 5-8) vs. LP (d 18-24)	<u>All results during quasi-LP:</u> *HC users only, pre-exposure:* • Increased BBT *Exercise:* • Increased T_c • **Increased sweating threshold** • **No phase differences in sweat gain**
Charkoudian & Johnson (1997)	• 7 untrained females • All	• Ramp heating of T_{sk} to 38.5°C for 30-45 min • Water perfused suit	<u>All results during high hormone phase</u> *Pre-exposure:*

	monophasic HC prescribed	• Subjects passive • Low hormone phase vs. high hormone phase (precise days undefined)	• Increased BBT *Heat exposure:* • Increased vasodilator threshold • Increased sweat threshold • **Decreased heart rate for a given T_c** • **Decreased sweat rate for a given T_c** • No phase differences in cutaneous vascular conductance slope • **No phase differences in sweat gain**
Tenaglia et al. (1999)	• 18 untrained females • 7 monophasic HC prescribed, 2 triphasic HC prescribed • 9 non-HC users	• Alternating 15 min walking with 15 min resting until volitional exhaustion in 40°C • Donned nuclear biological chemical ensemble • Quasi-FP (d 2-5)vs. quasi-LP (d 19-22) • FP (d 2-5) vs. LP (d 19-22)	<u>All results during quasi-LP</u> *HC users only, pre-exposure:* • Increased BBT *Heat exposure:* • Increased T_c • **No phase differences in tolerance time** • No phase differences in sweat gain <u>All results during LP:</u> *Non-HC users only, pre-exposure*: • Increased BBT • Increased T_{sk} *Heat exposure:* • Decreased tolerance time • Decreased ΔT_c • No phase difference in sweat gain
Armstrong et al. (2005)	• 36 untrained females • 9 monophasic HC prescribed, 6 triphasic HC prescribed • 7 injected contraceptive prescribed • 14 non-HC users	• 8 wk indoor heat acclimatization and outdoor physical training (HAPT) • FP (d 2-5) pre-HAPT vs. FP (d 2-5) post-HAPT	<u>All results during post-HAPT:</u> *HC users only:* • Decreased sweat threshold • Decreased time to sweat onset (minutes)

Often monophasic HC users serve as a control group in experimentation due to streamlined hormonal profiles; therefore, the majority of these participants were prescribed this type that could eradicate mid-cycle changes. In support, minimal differences were notied in sweat gain (Charkoudian & Johnson, 1997; Grucza et al., 1993). Interestingly, BBT and effector thresholds are similarly increased during the active phase in these individuals. The shifts are likely due to a natural tendency of the menstrual cycle that HC cannot supersede, and suggest a link between the thermoregulatory and reproductive systems that is susceptible to change without ovulation occurring. Unfortunately, these results cannot determine whether BBT and threshold alterations occur at the periphery. SP in the plasma may perfuse the blood vessels and sweat glands, plausible to modify the vasomotion or fluid output, respectively. It is also possible the synthetic hormones influence central drive to elevate response thresholds. Although equivocal, the latter hypothesis is generally agreed upon. Future studies should remain examining monphasic HC users, but isolate central from peripheral thermal information to better quantify thermal responses.

7) Gaps in the Literature

i) Methodological Challenges

Upon realizing little is clarified about female thermoregulation, many have applied thermal stress in combination with fluctuating sex hormones. Unfortunately, current knowledge is limited due to variable methods and subject characteristics, or inaccurate determination of the menstrual phase status. Changes in T_c involve the integration of central and peripheral thermal information (Simon, 2000), yet it is difficult to determine if the sex hormones affect the integration of the afferents, or solely central or peripheral control. Most suggest the effects are centrally occurring (Carpenter & Nunneley, 1988; Hesemer & Bruck, 1985), but findings are largely influenced by variations of T_{sk}. It cannot be clarified which thermal receptors are affected when effector gains are plotted as a function of body temperature derived from T_{sk} and T_c. Defining the intensity of thermal responses as a change in either T_{sk} or T_c alone, may eliminate

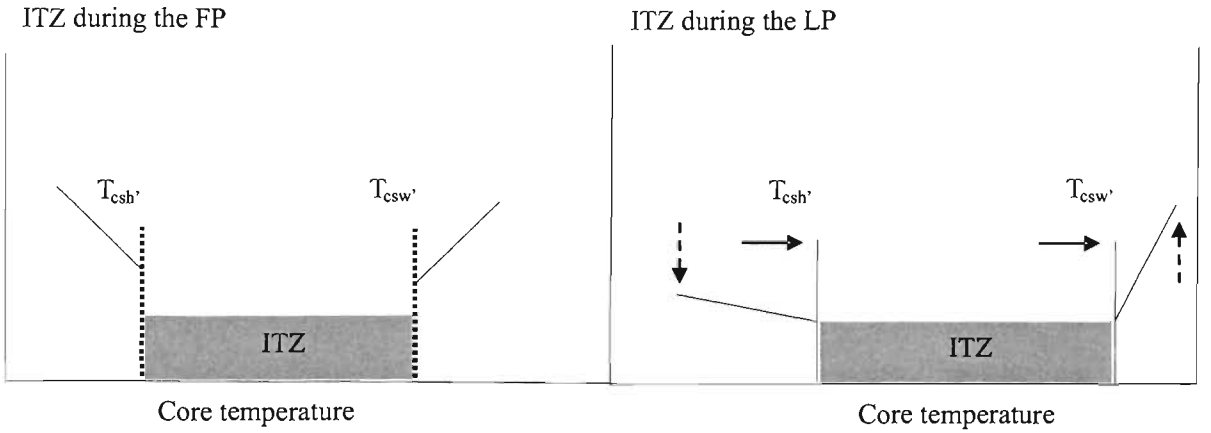
controversy and explain the mechanistics of thermoregulatory alterations. Unique findings may also be due to the time of day subjects were exposed to thermal stress. The study by Hessemer & Bruck (1985) was conducted between 03:00-04:30h when the BBT shift is most apparent. As well, the amplitude of circadian variation in T_c is less during the LP (Coyne et al., 2000), and thus warrants consideration.

The experimental conditions are widely variable between studies, allowing for different thermal inputs and potentiating discrepancy in effector gains. Subject fitness status ranged from adolescent gymnasts and military trained to untrained. Whether responses can be extrapolated for the entire female population is unclear. Furthermore, determining the menstrual phase was not always accomplished most accurately. For example, in Kenshalo (1966) the menstrual cycle phase was determined by charting BBT. Although ovulation is typically characterized by a sudden increase in BBT, this method relies on subject responsibility, and is susceptible to variability (Coyne et al., 2000). Lastly, studies examining HC users take varying pill types (monophasic and triphasic), and the dates of the hormone phases which they are tested in differ. In Grucza et al. (1997) quasi-FP testing occurred on days 9-10 of the menstrual cycle. In contrast, participants in the study by Charkoudian & Johnson (1999) were tested between days 1-7 during placebo ingestion, although still coined the quasi-FP.

ii) Sex Hormones and the Interthreshold Zone

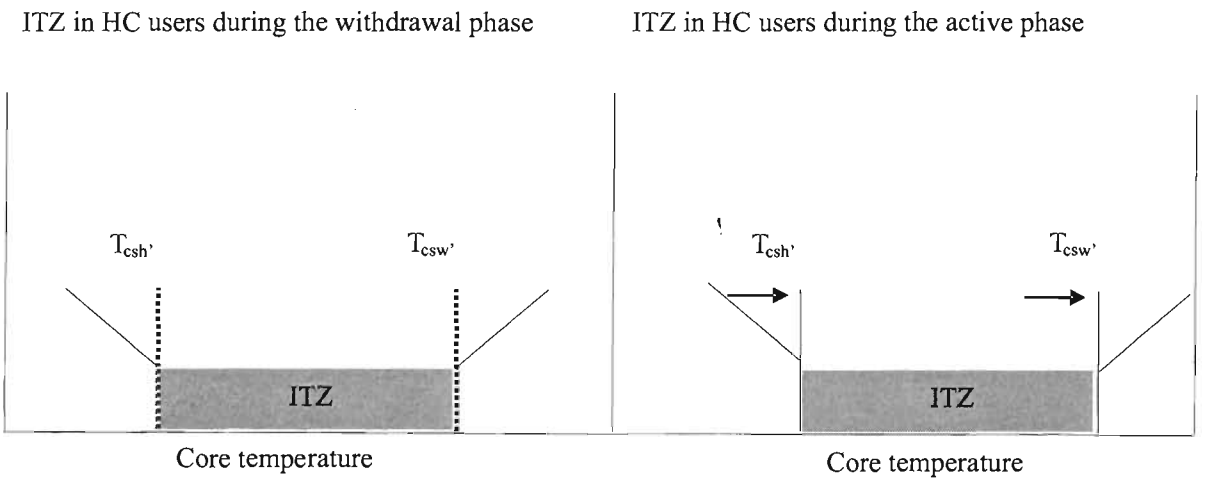
Despite methodological differences, current knowledge generalizes that during the LP or latter active phase, BBT is elevated in non-HC and HC users alike, respectively. Concomitant with this increase is an upwards shift in the thresholds for shivering and sweating. The literature does not agree whether effector gains decrease or increase, as only some report cold responses diminish, and hot responses augment to compensate for higher temperatures. Below (*Figure 2.7.*) are the replicated ITZ depictions that summarize the literature with respect to non-HC users, the ITZ, core shivering ($T_{csh'}$), and core sweating ($T_{csw'}$) thresholds as a function of T_c throughout the menstrual cycle.

Figure 2.7. The interthreshold zones between the follicular and luteal phases of the menstrual cycle in females not using contraceptives. The arrows indicate the alterations to the core temperature thresholds for shivering ($T_{csh'}$) and sweating ($T_{csw'}$). The dashed arrows depict the sweating and shivering gains potentially altered by the menstrual cycle phase status.



In HC users, phase differences may be dampened though this finding is inconsistent. Below in *Figure 2.8.* are the replicated ITZ depictions that summarize the literature with respect to HC users, the ITZ, $T_{csh'}$ and $T_{csw'}$ when plotted against T_c throughout the entire menstrual cycle.

Figure 2.8. The interthreshold zones between the active and withdrawal phases of the menstrual cycle in females using contraceptives. The arrows indicate the alterations to the core temperature thresholds for shivering ($T_{csh'}$) and sweating ($T_{csw'}$). In hormonal contraceptive (HC) users, no marked active phase differences in the shivering and sweating gains have been reported.



Rarely are cold and heat stress combined to test the same subject in variable environments. Noting how one person responds in both extremes will reduce variability, and better indicate how sex hormones affect thermal balance. As well, HC formulae vary not only between studies, but within subjects of a single study. Standardizing to one prescription type will render that group as a suitable control.

However, in order to better comprehend the central mechanism of PRO and OES that most likely occurs, it is worthy to monitor the T_c as it applies to the ITZ theory. A prior study by Anderson et al. (1995) replicated the protocol utilized previously by Mekjavic et al. (1991) to compare central effector thresholds between sexes. This model clamps T_{sk} through a stable water immersion protocol, thus removing the peripheral thermoafferents and monitoring solely central sweating and shivering thresholds (Mekjavic et al., 1991). The data found no significant sex differences in the rate of T_c cooling, nor sweating and shivering thresholds. Females did exhibit a decreased sweating gain that may be explained by sex hormones diminishing central sweat gland activation. More importantly, the females were only examined during one phase of the cycle, and thermoregulatory phase alterations disregarded. By utilizing a similar protocol wherein females are isolated and monitored throughout both phases of the menstrual cycle, findings may clarify the role of sex hormones on the central control of thermoregulation.

8) Summary

Although most of the literature proposes that OES and PRO affect a thermal set point, no study as of yet has utilized a protocol that assures this mechanism, nor indicates whether the width of the ITZ changes. The majority find an upwards shift during the LP in the activation thresholds for shivering and sweating, and that contraceptives dampen such shifts. On the whole, numerous hypotheses and inconsistent findings merit further research to better understand the effects of sex hormones on temperature regulation, and to accommodate a population that should be viewed separate from male counterparts.

Chapter 3- Objective and Hypotheses

9) Objectives

Literature suggests fluctuating sex hormones alter thermoregulatory response across the menstrual cycle and may account for sex differences to thermal stress. The research does not clarify whether hormones affect the central or peripheral thermophysiological pathways, or their integration. Research needs to better control core and skin temperatures to ascertain the role of progestogens and oestrogens in thermal responses. Therefore, the objectives of this study are to:

1. Determine the core thermoeffector thresholds in females using two different measurement sites,
2. Determine if the core thermoeffector thresholds are altered by the menstrual cycle and/or contraceptive use,
3. Determine if the thermoeffector gains are altered by the menstrual cycle and/or contraceptive use,
4. Determine if the width of the core interthreshold zone is altered by the menstrual cycle and/or contraceptive use.

10) Hypotheses

The hypotheses of this study are:

1. The thermoeffector thresholds will not be significantly different between the two different measurement sites used within a trial,
2. The core thermoeffector thresholds will increase during the luteal and active phases in non-contraceptive and contraceptive groups, respectively,

3. The sweating gain will be significantly increased, and the shivering gain will be significantly decreased during the luteal phase in the non-contraceptive group; however, neither the sweating nor shivering gains will be significantly different across the menstrual cycle in the contraceptive group,
4. The width of the core interthreshold zone will not be altered significantly in either group.

Chapter 4- Methodology

11) Participants

Inclusion criteria: 12 females were recruited based on age (18-40 years), menstrual cycle regularity (28-32 days), HC use, and body fat content (12-30%). After providing signed informed consent, subjects completed the following questionnaires to ensure health status and menstrual cycle normalcy (see *Appendix 4 and 5*):

- Modified Physical Activity Readiness Questionnaire (Thomas et al., 1992), and
- Menstrual history questionnaire.

Subjects who passed preliminary health screening were divided into two groups:

- Not using any exogenous contraceptive preparation for at least 3 mo (NCG, n=7)
- Using the same exogenous monophasic contraceptive preparation for at least 3 mo (HCG, n=5).

Exclusion criteria: any females that exhibited a potential eating disorder, chronic health condition (diabetes, cardiovascular disease, etc.), contraindication to exercise or menstrual dysfunction were disqualified from participation. Menstrual cycle dysfunctions included oligomenorrhea (a menstrual cycle lasting longer than a 42 days), menorrhagia (greater than 7 days of heavy blood loss during menstruation), and amenorrhea (absence of menstruation for 6 months or longer).

For all trials, subjects were outfitted in a two-piece swim suit, and were requested to avoid strenuous exercise, caffeine, and alcohol consumption 24 hr prior. A light meal representative of normal diet consumption was allowed approximately 2 hr prior. Subjects provided a urine sample to ensure adequate hydration before each trial. Participation for that day was void if urine specific gravity exceeded a value of 1.020.

12) Experimental Design

This study required subjects to participate in the following two trials:

i. Trial 1

The first trial (Trial 1) took place during menstruation or the first week of high hormone dose for the NCG and HCG, respectively (days 2-5). Once assigned to the appropriate group, height, weight, body composition (as according to Jackson, Pollack and Ward (1980)), and basal $\dot{V}O_2$ were measured. Subjects donned a Hans Rudolph mask connected to an online gas collection system (Moxus, AEI Technologies) and rested for 10 min. The average of the $\dot{V}O_2$ values obtained during min 9-10 was used as the resting $\dot{V}O_2$ for trial 1. Maximal HR was then predicted based on the following regression equation by Tanaka, Monahan & Seals (2001), and used to determine the relative workload:

$$208\text{-}0.7(\text{age}).$$

During instrumentation, subjects donned a terrycloth bathrobe and fleece slippers to remain warm while clad in only swimsuits. Following set-up and transfer into an immersion tank, subjects underwent head out immersion in water maintained at 28±0.5°C. After a 1 min rest period, subjects performed exercise on an underwater cycle ergometer for 20-30 min or until steady-state sweating was achieved (Anderson et al., 1995). The first 10-20 min of exercise was at an intensity of 60-70% of their estimated maximal HR, followed by 10 min of intervals alternating 30 sec maximal effort and 30 sec rest. Subjects then remained seated on the ergometer in the water until $\dot{V}O_2$ doubled from resting value (Mekjavic et al., 1991). During cooling in both trials, the water in the tank was continuously agitated to maintain convective heat exchange.

Rectal temperature (T_{re}), tympanic temperature (T_{ty}), mean skin temperature ($\overline{T}_{sk}$), HR, SR, relative oxygen consumption ($\dot{V}O_2 \bullet kg^{-1}$), and blood perfusion (PU) were monitored every 30 sec with appropriate acquisition software. Thermal comfort and thermal sensation (Cabanac, 1979) were also recorded at 5 min intervals.

ii. Trial 2

The second trial (Trial 2) involved identical subject preparation, experimental protocol, and measurement collection, however took place 4-7 days following ovulation in the NCG, and during days 18-21 of the high hormone dose in the HCG. Due to daily influences on basal $\dot{V}O_2$, the average $\dot{V}O_2$ value was again obtained during min 9-10 rest upon arrival to the laboratory using the same gas collection system as trial 1. If a NCG participant experienced an anovulatory cycle (negative ovulation test or PRO content <3 μg/mL in the LP), both trials 1 and 2 were repeated following the successive menstrual period so data was collected from the same menstrual cycle.

13) Determination of the Menstrual Cycle

Before data collection, NCG participants tracked the 3 menstrual cycles prior to trial 1 to provide investigators an estimated length of the menstrual cycle. These subjects also provided daily urine samples that were analyzed for urinary concentrations of hormone metabolites: estrone 3-glucuronide (E1G), and pregnanediol 3-glucuronide (PdG). From these samples, hormonal profiles of E1G, and PdG were determined. Participants were provided with labelled specimen cups to collect the first morning void. Subjects temporarily stored samples in the refrigerator (4°C) for no longer than 7 days until delivery to the laboratory. Samples were aliquoted and stored at -20°C prior to analysis carried out by immunoassay (Munro et al., 1991). To confirm that ovulation occurred, NCG were provided with ovulation strip kits (test strip format, Kurkel Enterprises, Bellingham, WA). The strips were placed in the urine samples beginning on day 12 of the menstrual cycle as instructed in each kit. An ovulation test was done until a positive result to determine the date for trial 2.

Participants in the HCG group were not required to track consecutive menstrual cycles or conduct ovulation tests, due to consistent menstruation and prevented ovulation. Upon awakening on the designated experimental day however, subjects were supplied with two collections cups to sample first

morning voids, and brought upon arrival to the laboratory. These were analyzed by the same immunoassay for E1G and PdG concentrations.

14) Measurements

Immersion tank

The immersion tank had a diameter of 74" and a height of 60." The water temperature was maintained at 28°C using a heater (K-Star, Woodridge, ON) and chiller (A-Struyk, Dunnville, ON) accurate to ± 0.5°C, and continuously stirred by use of a pump (Hayward Super Pump, Oakville, ON).

Body temperatures

Participants had T_c measured by T_{re} and T_{ty} temperatures. Subjects self-inserted a flexible rectal probe (Mon-A-Therm Core, Mallinkrodt Medical, St. Louis, USA) 12 cm past the anal sphincter. T_{ty} was monitored with a specialized, flexible thermistor (Mallinkrodt Medical, St. Louis, USA) self-inserted into the ear canal. The probe was secured using cotton balls strategically placed and adhered with surgical tape (3M Transpore Tape, 3M Canada). To measure $\overline{T}_{sk,}$ 7 heat flow transducers embedded with thermistors (FR-025-TH44033-F6, Concept Engineering, Old Saybrook, CT) were securely attached to the skin with surgical tape and stretch gauze (Derma Duform Conforming Bandage). $\overline{T}_{sk}$ was calculated formulating a mean of the 7 sites through DasyLab Data Acquisition System Laboratory software (Version 10.0, Measurement Computering, Norton, MA) by the following equation according to Hardy & DuBois (1938):

$$\overline{T}_{sk} = 0.07(\text{forehead}) + 0.14(\text{forearm}) + 0.05(\text{hand}) + 0.07(\text{foot}) + 0.13(\text{shin}) + 0.19(\text{quad}) + 0.35(\text{abdomen}).$$

Using $\overline{T}_{sk}$ and T_{re}, a two-compartment model mean body temperature (T_b) was calculated using the following equation, as according to (Lenhardt & Sessler, 2006):

$$T_b = x \cdot T_{re} + (1 - x)\,\overline{T}_{sk}, \text{ so that } T_b = 0.64\,T_{re} + 0.36\,\overline{T}_{sk},$$

where 0.64 = x, and 0.36 = 1 – x. All body temperature measurements were recorded every 30 s using a data logger (Smartreader 8 Plus, ACR, Vancouver, Canada) connected to a computer for continual monitoring.

Heart rate

Subjects were fitted with a heart rate monitor (s810i, Polar Electro Oy, Finland) placed around the thorax. The electrodes within the plastic transmitter recorded real-time heart rate data from exercise and cooling.

Sweat rate

A ventilated sweat capsule was firmly attached with Skin Preparation Wipes (Smith and Nephew Protective Barrier Wipes) and spirit gum (Graftobian, Madison, WI) to the forehead, on the lateral side of the frontal bone to measure local sweat rate ($mg \cdot cm^{-2} \cdot min^{-1}$). A thin, nylon/spandex toque was also worn on the head to ensure local sweating during immersion exercise. SR was determined by quantifying the difference in air temperature and density entering and leaving the capsule using a temperature and humidity sensor (Omega HX93, Omega Engineering, Stanford, CT). The air entering the capsule was dry and of a known, constant volume (Brooks 5850, mass flow controller, Emerson electric, Hetfield, PA), flowing at a rate of 0.25 $L \cdot min^{-1}$.

Oxygen uptake

$\dot{V}O_2$ and rate of carbon dioxide exhalation was determined from analysis of expired air. The air was directed to and analyzed in an online gas collection system (Moxus, AEI Technologies), collected breath-by-breath, and averaged over 30 s intervals.

Skin blood perfusion

Forehead blood flow was measured using a laser Doppler velocimetry system (Periflux System 5000, main control unit; PF5010 LDPM, function unit; Perimed, Stockholm, Sweden). The Doppler flow meter required calibration using the appropriate Perimed Doppler calibration unit. The small probe (PR 407 Small Straight Probe, Perimed) was secured to the forehead with surgical tape (3M Transpore Tape, 3M Canada). The blood flow to the region was expressed in PU and recorded every 30 sec.

Urine samples

To determine urine specific gravity, samples were placed on a refractometer lens (Atago, PAL-10S, USA) following calibration using distilled water.

15) Data Analysis

The resulting changes from resting T_{re} and T_{ty} were used for determining the $T_{csw'}$, $T_{csh'}$ and ITZ width. To determine the slopes of the effector responses, SR, $\dot{V}O_2$, and skin blood perfusion were plotted against T_c. The shivering gains were attained from the linear slope of the line averaging the data 5 min pre- and post- shivering threshold ($\dot{V}O_2/\Delta^{\circ}C^{-1}$). Sweat gains ($SR/\Delta^{\circ}C^{-1}$) were collected from the data at exercise termination until the $T_{csw'}$. The intensity of vasomotor activity within the ITZ was averaged and expressed as $PU/\Delta^{\circ}C^{-1}$. To compare the thermoeffector thresholds and gains within menstrual phase trials and between NCG and HCG groups, a 2-way mixed design ANOVA with repeated measures was conducted. Significance was reported at a level of $P < 0.05$. All analyses were performed with SPSS statistical software package (Version 16.0).

Figure 4.1. Throughout cooling, the thresholds for the sweat (SR) and shiver (VO2) responses will correspond with a core temperature value, thus providing the width of the core interthreshold zone as well. The gains of the sweat, shiver and skin blood flow (PU) responses are also identified, and represented by theta (θ).

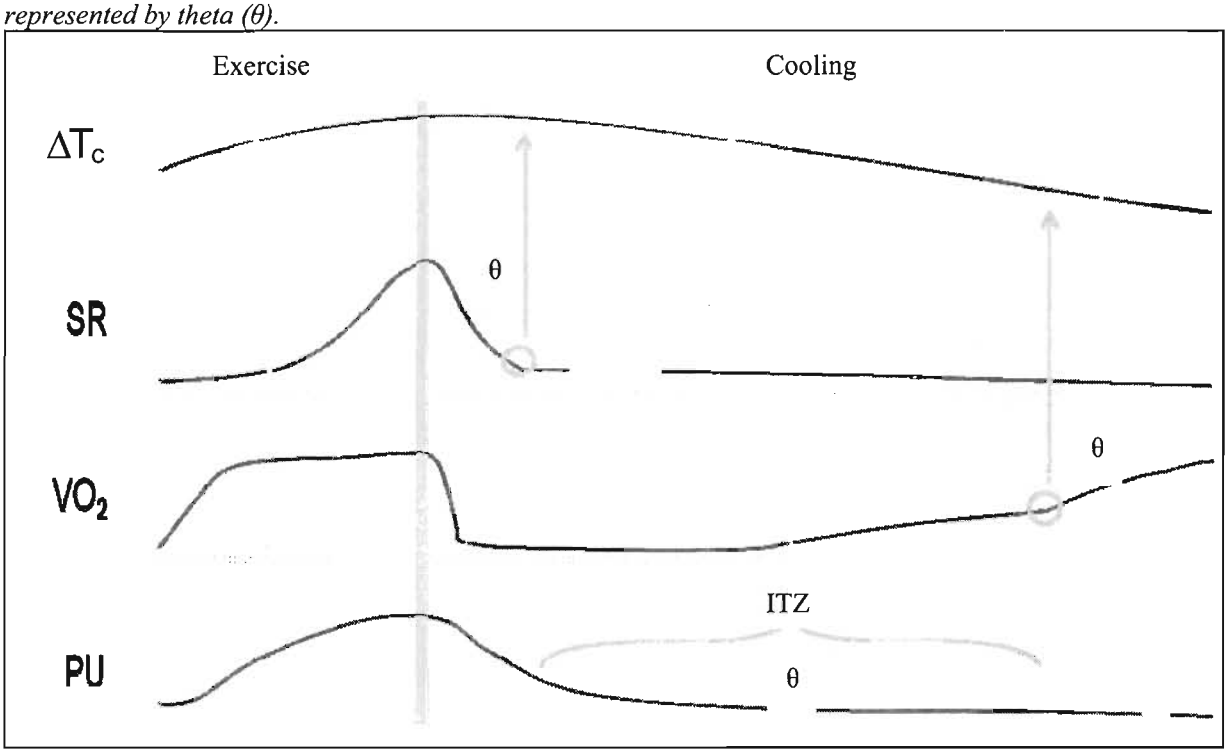
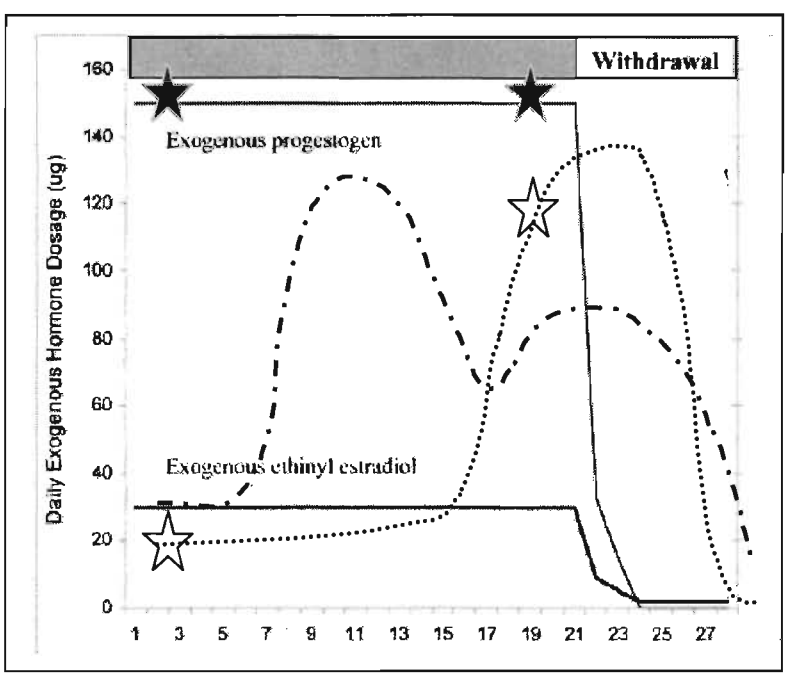


*Figure 4.2. Hypothesized fluctuations of endogenous and exogenous hormones during one month dose in the hormonal contraceptive group (**solid** lines, HCG) (from Rechichi et al., 2009) or one menstrual cycle in the non-contraceptive group (**dotted** lines, NCG). The HCG withdrawal phase is indicated. Stars denote approximate time of testing for trials 1 and 2 to better compare progestogen content between groups and trials.*



For NCG only:
Endogenous oestradiol - - - -
Endogenous progesterone .....

Note: Hormonal concentrations not drawn to scale between groups.

Chapter 5- Results

1) Subject demographic profiles

Table 5.1. summarizes the characteristic data for both groups (NCG, HCG). The NCG participants were significantly older than the HCG participants ($P<0.05$). No other significant differences were found in the remaining dependent variables. The means, standard deviation (± SD), and ranges are provided.

Variable	NCG	Range NCG	HCG	Range HCG
Age (y)	31.66 ± 5.88‡	26-42	23.60 ± 1.94	21-26
Height (cm)	169.45 ± 5.76	163.0-180.0	169.14 ± 6.38	165.7-180.5
Weight (kg)	68.94 ± 10.04	56.20-80.18	64.87 ± 6.15	61.98-73.4
Relative body fat percent	19.61 ± 4.71	13.9-27.1	17.98 ± 4.24	15.2-25.5
Body surface area (m^2)	1.82 ± 0.13	1.59-1.99	1.74 ± 0.09	1.63-1.86
Surface area to mass ratio (cm^2/kg)	2.67 ± 0.30	2.45-3.24	2.69 ± 0.14	2.47-2.85
Approximate menstrual cycle length (d)	28.16 ± 2.56	25-32	28 ± 0	28 - 28

Table 5.1. Physical characteristics and menstrual cycle lengths for the non-contraceptive (NCG; n=7) and hormonal contraceptive (HCG; n=5) groups. ‡ Significantly different from HCG group ($P<0.05$).

2) Hormonal contraceptive type

Table 5.2. provides the monophasic contraceptive type, and synthetic oestrogen/progestin concentrations in each dosage for the HCG.

Subject code	Hormonal Contraceptive Brand	Synthetic oestrogen	Synthetic progestin
BD	Marvelon 21	Ethinyl oestradiol 0.03 mg	Desogestrel 0.15 mg
CG	Yasmin	Ethinyl oestradiol 0.03 mg	Drospirenon 3.0 mg
ED	Marvelon 21	Ethinyl oestradiol 0.03 mg	Desogestrel 0.15 mg
LC	Marvelon 28	Ethinyl oestradiol 0.03 mg	Desogestrel 0.15 mg
ND	Evra	Ethinyl oestradiol 0.60 mg	Norelgestromin 6.0 mg

Table 5.2. Individual monophasic contraceptive brand names and synthetic hormonal doses.

3) Hormonal profiles

One NCG participant experienced 2 consecutive anovulatory cycles thus was excluded from participation. This participant's data is not included in *Table 5.1.* Three participants (2=1 NCG; n=1 HCG) performed trial 2 first, then trial 1 due to scheduling restraints. Therefore, the estrone 3-glucuronide (E1G) and pregnanediol 3-glucuronide (PdG) data were obtained from different, but consecutive menstrual cycles. All other participants' hormonal data were analysed from the same menstrual cycle that the trials took place. The HCG E1G and PdG data were assumed constant between trials due to the monophasic nature of the contraceptive prescribed. NCG participants collected daily urine samples that were analysed in retrospect, beginning from the first or second day of menstruation until trial 2, 4-8 days post-positive ovulation. As expected, the NCG had significantly higher PRO content during trial 2 than trial 1 ($P<0.05$), as well as significantly greater PRO content during trial 2 compared to the HCG ($P<0.01$). *Table 5.3.*

presents the group E1G and PdG data analysed by enzyme immunoassay. The means, and standard deviation (± SD) are provided.

Group	E1G ($ng \cdot ml^{-1}$)		PdG ($\mu g \cdot ml^{-1}$)	
	FP	LP	FP	LP
NCG (7)	28.44 ± 13.72	38.56 ± 12.15‡	1.44 ± 0.99	6.94 ± 4.8*‡
HCG (5)	15.25 ± 11.62	15.38 ± 6.91	0.64 ± 0.33	0.55 ± 0.28

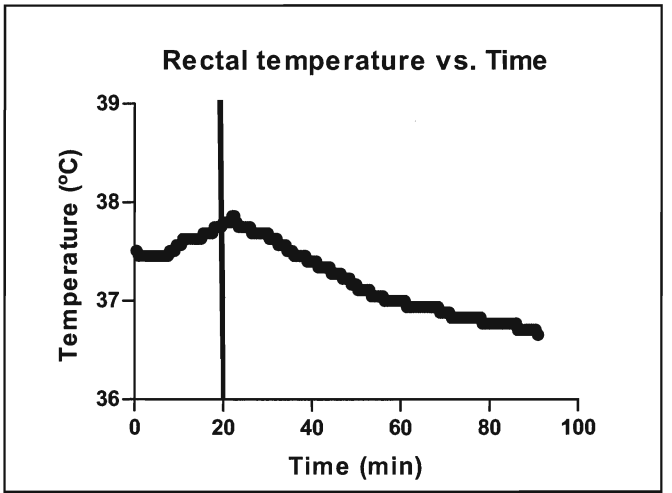
Table 5.3. Number of subjects in parentheses. Basal estrone 3-glucuronide (E1G) and pregnanediol 3-glucuronide (PdG) content from non-contraceptive (NCG) and hormonal contraceptive (HCG) groups. Urinary metabolite data was collected on the days of the experiments during both trials. Trial 1 testing for NCG took place on days 2-5, and trial 2 testing 4-8 days post-positive ovulation. Trial 1 testing for HCG took place on days 2-5 of high hormone dose, and days 18-21 of high hormone dose for trial 2. ‡Significantly different from HCG group ($P<0.01$).*Significantly different from trial 1 ($P<0.05$).

4) Core temperature and skin temperature responses

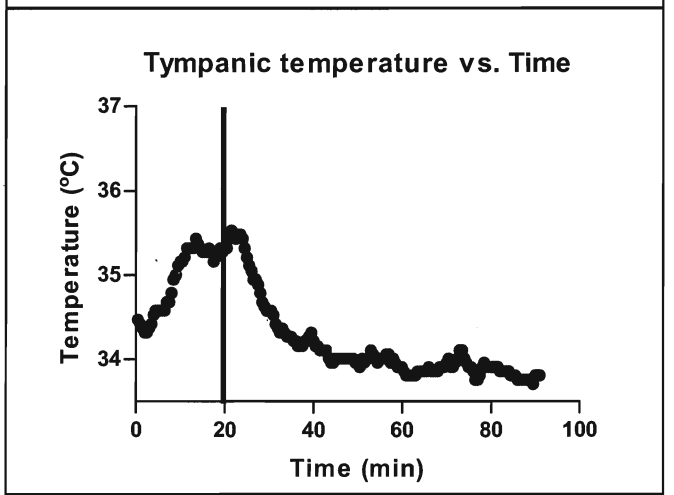
An independent *t-test* revealed that basal temperature, all thermoeffector threshold temperatures, and cooling rate (trial 2 only) were significantly different between T_{re} and T_{ty} sites ($P<0.001$). This significant finding will be elaborated on in the discussion. *Figure 5.1 (A-C)* illustrate typical body temperature responses from one HCG participant across time. Even though exercise duration varied between subjects because of variable sweating responses, thermal responses were relatively similar between all subjects (see *Appendix*). The 2 (phase) x 2 (group) mixed-model ANOVA revealed no interaction effect. There were significant differences in basal T_{re}, but the HCG had significantly lower basal T_{ty} ($P<0.01$) regardless of trial (HCG: trial 1 34.60 ± 0.44°C, trial 2 34.59 ± 0.27°C; NCG: trial 1 35.01 ± 0.36°C, trial 2 35.09 ± 0.21°C). In both groups, T_c increased due to exercise as anticipated. The magnitude of T_{re} increase was almost higher ($P=0.08$) for trial 2. For the NCG, T_{re} and T_{ty} increased due to exercise 0.39 ± 0.29°C and 1.37 ± 0.21°C respectively for trial 1, 0.59 ± 0.39°C and 1.39 ± 0.55°C for trial 2. Presented in the same fashion for the HCG, the increases were 0.43 ± 0.22°C and 1.18 ± 0.82°C, and 0.69 ± 0.28°C and 1.29 ± 0.42°C. During the cooling portion of the trial, T_{re} and T_{ty} slowly declined, but showed vast inter-individuality. For instance, some participants exhibited greater post-exercise T_{re}

increases than others, due to the response lag of this measurement site (Kenny et al., 1999). In addition, T_{re} increases concomitant with sweat rate (SR) decreases contributed to variable sweat response gains. Overall, only the rate of T_{re} cooling differed between groups ($P<0.05$), with HCG participants cooling quicker (trial 1 -1.15 ± 0.43°C, trial 2-1.00 ± 0.50°C) versus NCG participants (trial 1 -0.58 ± 0.22°C, trial 2 -0.52 ± 0.29°C). Rates of cooling rendered insignificant when measured by T_{ty}. $\overline{T}_{sk}$ was not significantly altered by menstrual phase or group, thus, the intent of the water immersion to stabilize $\overline{T}_{sk}$ was achieved.

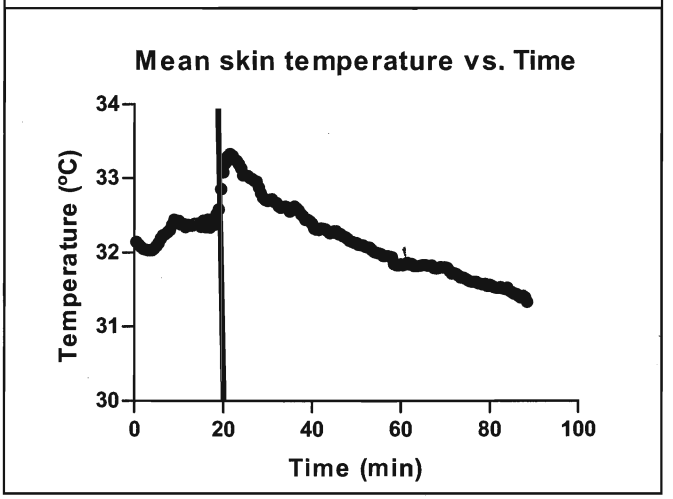
A.



B.



C.



Figures 5.1. Rectal (*A*), tympanic (*B*), and mean skin (*C*) temperatures during exercise and post-exercise cooling from one HCG participant. These recordings are representative of all subjects. The black line represents the end of exercise.

5) Metabolic rate and oxygen consumption

$\dot{V}O_2$ and other respiratory measures were obtained to calculate basal metabolism. Results found no interaction effect for this dependent variable. There was a trend (P=0.06) for metabolic rate to be higher in trial 2. During exercise, $\dot{V}O_2$ increased, and then quickly stabilized upon completion. Due to the water bath extracting heat energy from the participants' bodies, $\dot{V}O_2$ increased with the incidence of tremors, allowing the core temperature shivering thresholds to be identified (*Figure 5.2.*).

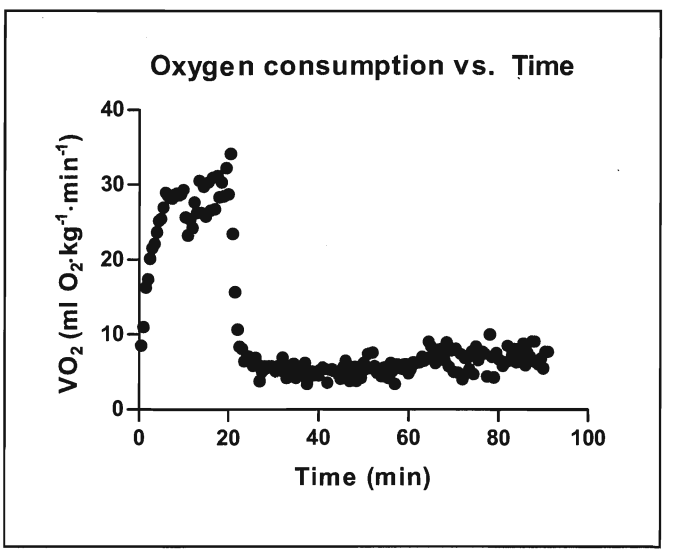


Figure 5.2. Respiratory response due to exercise (0 to 20 min) and cooling (20 to 90 min) from the same HCG participant whose data is illustrated in *Figure 5.1 (A-C)*. These recordings are representative of all subjects.

6) Sweat response

Steady-state sweating was achieved in all individuals due to exercise. As a result of individual variability in SR, some participants required 30 min of bicycling while others only 20 min. Thereafter, SR declined after exercise ended until evaporative heat loss terminated completely, and identified the cessation threshold (*Figure 5.3.*).

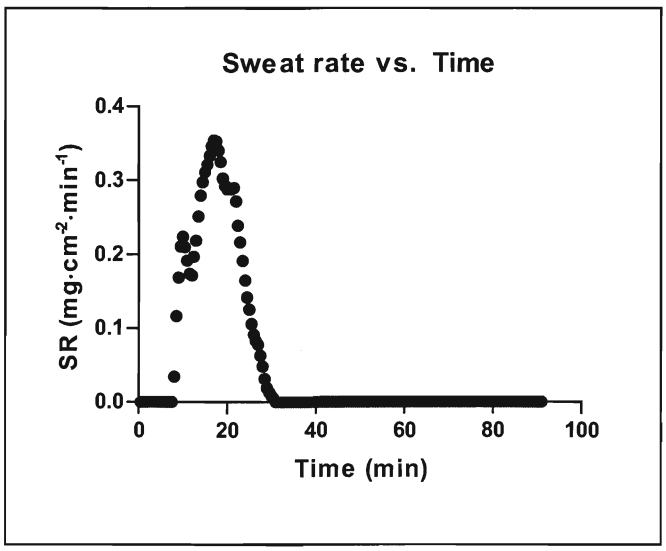


Figure 5.3. Sweat response due to exercise (0 to 20 min) and cooling (20 to 90 min) from the same HCG participant whose data is illustrated in *Figure 5.1 (A-C)*. These recordings are representative of all subjects.

7) Thermoeffector thresholds

The central thermoeffector thresholds were defined at the cessation of sweating, and onset of shivering. When the effector activity satisfied threshold criterion, both the absolute and relative changes from basal T_{re} and T_{ty} were analysed. Using the mixed-model ANOVA, there was no interaction effect. However, the absolute rectal sweat cessation threshold (T_{resw},) and relative rectal sweat cessation threshold (ΔT_{resw},) were significantly greater in trial 2 ($P<0.05$). At the tympanic site, the absolute sweat cessation threshold (T_{tysw},) was significantly elevated for the NCG versus the HCG ($P<0.05$), but indifferent between trials. With respect to the shiver thresholds, results showed no significant differences between groups or within trials ($P>0.05$) in neither absolute (T_{resh},/T_{tysh},) nor relative (ΔT_{resh},/ΔT_{tysh},) shivering thresholds. The series of data in *Table 5.4.* illustrate the central thermoeffector threshold temperatures for both groups during the two experiments.

8) Interthreshold zone widths

Within the sweat cessation and shivering onset thresholds is the T_c range that maintains thermoneutral through changes in vascular tone, coined the interthreshold zone (ITZ). There was no

interaction effect for ITZ width. Provided that sweat thresholds were elevated during trial 2, the width of the rectal ITZ in this trial was significantly larger ($P<0.05$). The tympanic ITZ width was nearly different between experiments ($P=0.10$), and elicited a significant group main effect ($P<0.05$), whereby NCG participants had a larger gap between effector activity. The series of data in *Table 5.4* display the group thresholds and ITZs. Following in *Figure 5.4. (A-B)*, the group thresholds and ITZs are depicted in vertical bar graphs. Below these, in *Figure 5.5. (A-B)* and *Figure 5.6. (A-B)* are a comparison of the thermoeffector thresholds and the resultant ITZs between trials in the same HCG participant whose data was used in all the above.

T_{re}	$T_{resw'}$ (°C)	$T_{resh'}$ (°C)	$\Delta T_{resw'}$ (°C)	$\Delta T_{resh'}$ (°C)	ITZ (°C)
Trial 1	37.55 ± 0.39	36.91 ± 0.50	0.15 ± 0.45	-0.49 ± 0.47	0.64 ± 0.22
Trial 2	37.90 ± 0.46*	37.07 ± 0.45	0.50 ± 0.38*	-0.75 ± 0.83	0.82 ± 0.37*

Table 5.4A. All absolute and relative sweat thresholds ($T_{tysw'}/\Delta T_{tysw'}$), shivering thresholds ($T_{tysh'}/\Delta T_{tysh'}$), and interthreshold zones (ITZ) for rectal temperature (T_{re}) between trials 1 and 2. Data presented as means ± SD. * Significantly different from FP ($P<0.05$).

T_{ty}	$T_{tysw'}$ (°C)	$T_{tysh'}$ (°C)	$\Delta T_{tysw'}$ (°C)	$\Delta T_{tysh'}$ (°C)	ITZ (°C)
Trial 1	35.20 ± 0.77	34.27 ± 0.63	0.37 ± 0.62	-0.55 ± 0.46	0.92 ± 0.40
Trial 2	35.66 ± 0.87	34.11 ± 0.85	0.80 ± 0.65	-0.75 ± 0.83	1.52 ± 0.84

Table 5.4B. All absolute and relative sweat thresholds ($T_{tysw'}/\Delta T_{tysw'}$), shivering thresholds ($T_{tysh'}/\Delta T_{tysh'}$), and interthreshold zones (ITZ) for tympanic temperature (T_{ty}) between trials 1 and 2. Data presented as means ± SD.

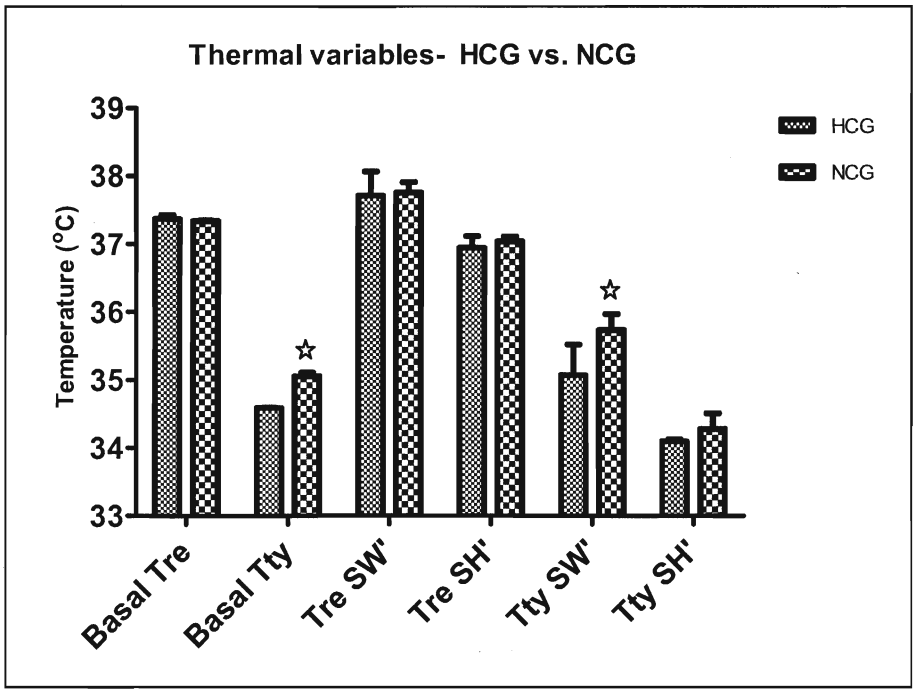


Figure 5.4. Hormonal contraceptive group (HCG) versus non-contraceptive group (NCG) absolute basal temperatures, sweat thresholds ($T_{resw'}$, $T_{tysw'}$), and shivering thresholds ($T_{resh'}$, $T_{tysh'}$) for both trials. Data presented as means ± SD. ☆ Significantly different from HCG ($P<0.05$).

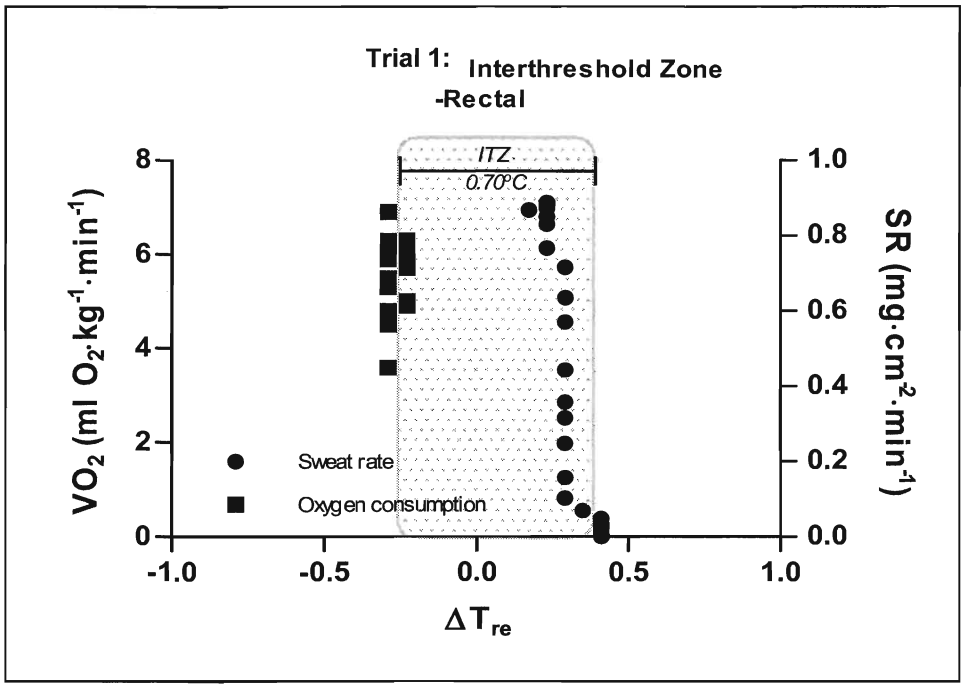


Figure 5.5A Interthreshold zone (ITZ) depiction for the rectal temperature (T_{re}) site during trial 1. These recordings are representative of all subjects.

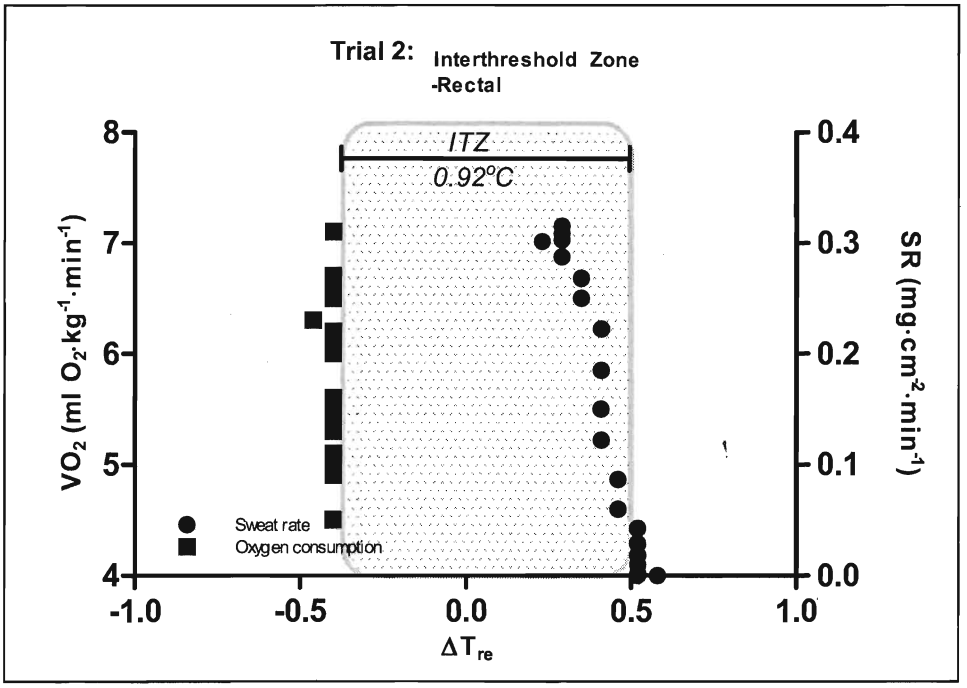


Figure 5.5B. Interthreshold zone (ITZ) depiction for the rectal temperature (T_{re}) site during trial 2. These recordings are representative of all subjects.

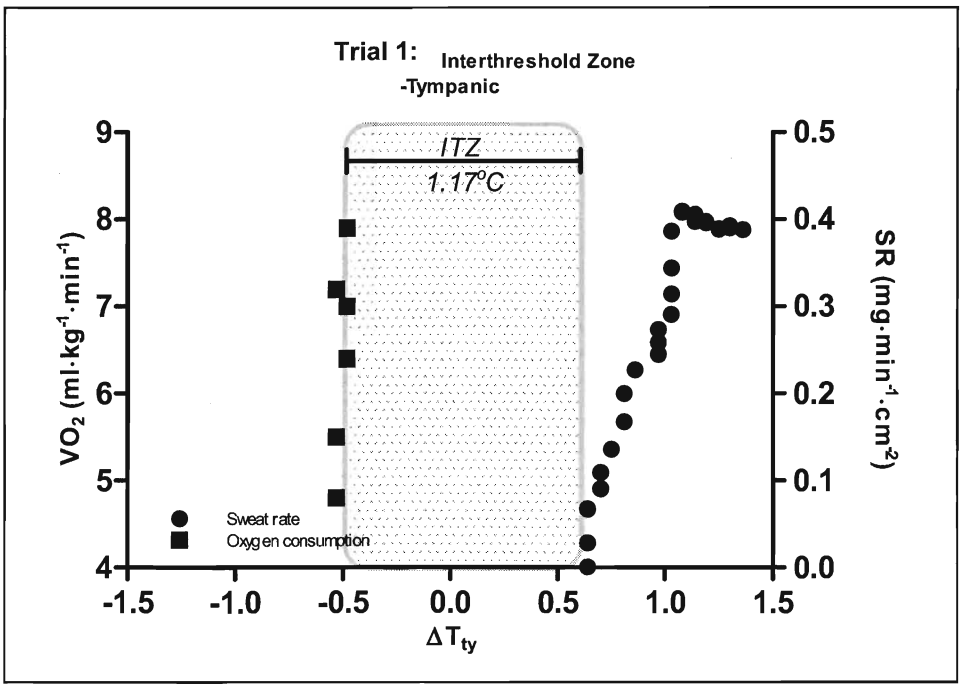


Figure 5.6A. Interthreshold zone (ITZ) depiction for the tympanic temperature (T_{ty}) site during trial 1. These recordings are representative of all subjects.

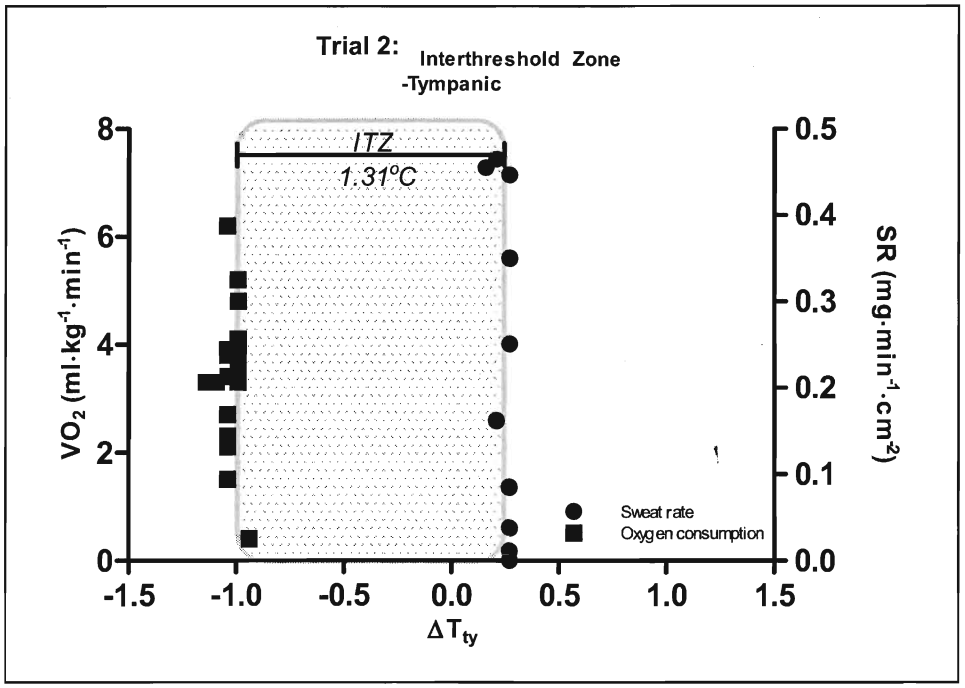


Figure 5.6A. Interthreshold zone (ITZ) depiction for the tympanic temperature (T_{ty}) site during trial 2. These recordings are representative of all subjects.

9) Thermoeffector gains

Linear regressions were conducted on thermoeffector activity plotted against changes in T_{re} and T_{ty}. For all participants, the intensity of the SR response was determined using the data obtained between exercise termination and sweat cessation. Meanwhile, the intensity of the $\dot{V}O_2$ response was determined using the data 5 min pre- and 5 min post-shivering onset. The ANOVA results found no significant interaction, but a trend (P=0.062), wherein shivering intensity decreased during trial 2 in NCG participants, but increased during trial 2 in HCG participants. As well, a near significant phase main effect (P=0.10) of a greater SR gain during trial 2 was also noted.

10)Forehead blood flow

During exercise, forehead blood flow (FBF) increased substantially to enable convective and conductive heat transfer. During cooling, FBF decreased as a result of vasoconstriction, and preserved T_c. This typical response is depicted in *Figure 5.7*. The laser Doppler measurement acquisition and arbitrary perfusion units (PU) do not allow absolute measures of FBF to be quantified. Instead, the linear decrease in FBF from sweat cessation to shiver onset was determined using regression, and provided the intensity of vasomotor tone required to maintain thermoneutraul within the ITZ. There was no interaction effect. As well, the declination slope of FBF throughout time was not statistically different, but drifted towards significance by both group (P=0.17), and phase (P=0.10). The HCG exhibited greater FBF gain versus the NCG, while the change in vasomotor tone was greater during cooling in trial 2.

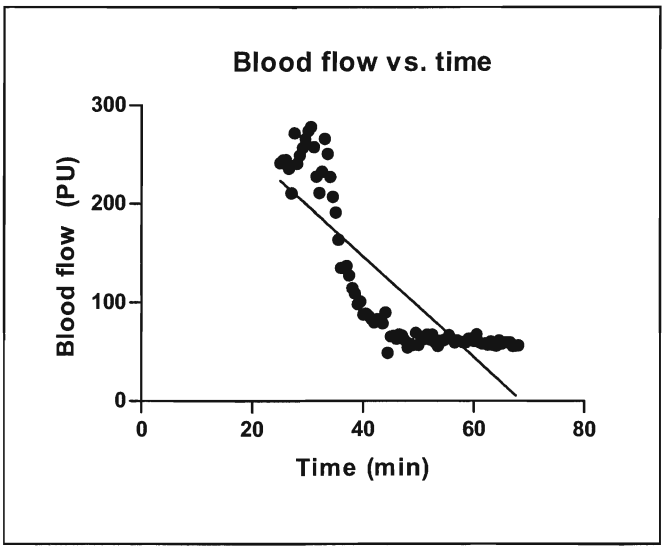


Figure 5.7. Blood flow response due to cooling after exercise (20 to 90 min) from the same HCG participant whose data is illustrated in *Figure 5.1 (A-C)*. These recordings are representative of all subjects.

11)Trial length and thermal votes

Even though T_{re} cooling rates varied between groups, the total length of water immersion was insignificantly different ($P>0.05$), and there was no interaction effect. During trial 1, the trial lengths were slightly longer, yet not statistically ($P=0.19$). The subjective measures at the time of shivering were not significantly altered due to menstrual phase ($P>0.05$), but TC was ($P<0.05$), and TS nearly ($P=0.093$) statistically different between groups. HCG participants perceived the cold stress as significantly less comfortable, and made them closer to unbearably cold. The TC and TS votes are illustrated below in *Figure 5.8.* in attempt to quantify whole body thermal "pleasure" and "comfort level" throughout cooling.

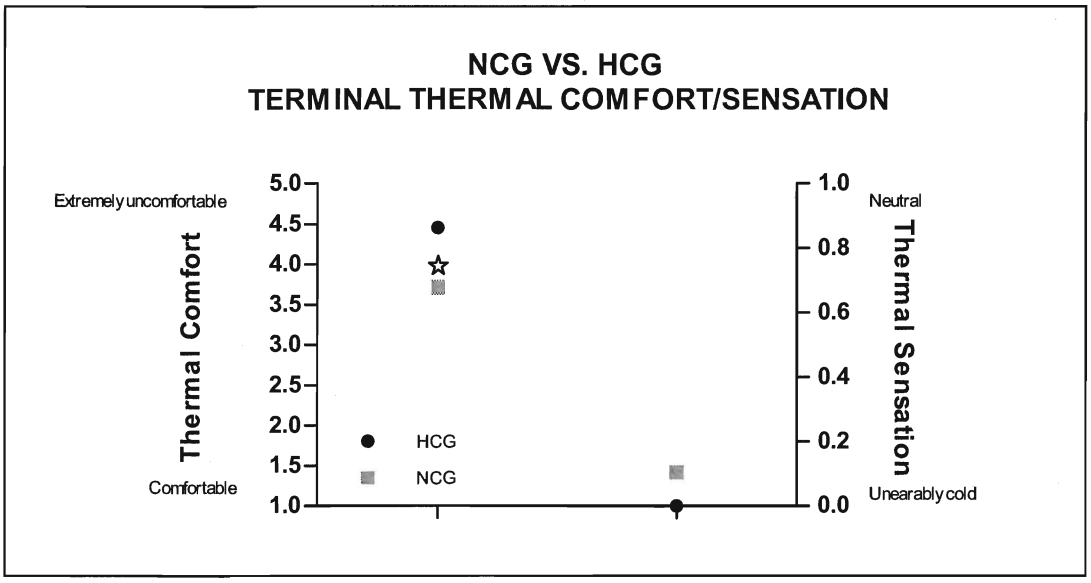


Figure 5.8. Hormonal contraceptive group (HCG) versus non-contraceptive group (NCG) thermal comfort (TC) and thermal sensation (TS). ☆ Significantly different from HCG ($P<0.05$).

Chapter 6- Discussion

In the present study, the major findings were that in both groups, basal body temperature was unchanged pre- versus post-ovulation. However, the central sweat cessation threshold was elevated during trial 2 when measured at the rectal temperature site. As a result, the width of the ITZ between sweating and shivering thresholds was larger with respect to T_{re} only. The HCG cooled more quickly post-exercise compared to the NCG, and elicited a narrower ITZ when measured at the tympanic temperature site. In addition, the 2 different sites that measured T_c were not similar, providing inconsistent differences in the thermal variables depending on which site was utilized.

Previous studies suggest that BBT increases approximately 0.3-0.5°C (Kolka & Stephenson, 1989; Frascarolo et al., 1990) post-ovulation due to a rise in PRO concentration (Hessemer & Bruck, 1985; Kolka & Stephenson, 1989), though the mechanism linking the two events remain unclear. In anaesthetized animals, PRO caused the discharge rate of cold-sensitive neurons in the hypothalamus to increase (Nakayama et al., 1975); thus, activation of these neurons initiates cold thermoeffector responses and may increase BBT. To add to the ambiguity, other mechanisms include increased non-shivering thermogenesis due to an elevated basal metabolic rate or an infection-inducing role of PRO that increases interleukin-1 secretion from immune cells (Cannon & Dinarello, 1985; Saladin, 2001). Against the general consensus, subjects in either group did not display a significant change in BBT throughout trials. One could argue that the NCG participants were anovulatory, but LH surge detection kits were provided, and all participants provided positive test results. When BBT is increased post-ovulation, it is known as a biphasic pattern; however, some women ovulate without this distinct BBT change to create a monophasic pattern (Barron & Fehring, 2005). Inspection of the data shows that out of all 7 ovulating NCG participants, only 2 elicited biphasic patterns. This is further supported by pilot BBT collection over 2 consecutive menstrual cycles (n=3) that did not detect mid-cycle BBT increases (data not shown). 2 HCG participants also exhibited biphasic patterns, as the natural tendency of the menstrual cycle overrides even

though ovulation is prevented (Grucza et al., 1997). There are many factors known to affect BBT—alcohol consumption, late hours of wakefulness, oversleeping, travel, time zones, stress, illness, and medications—many of which were controlled as best possible, but cannot be ruled out entirely. Even more so, Barron & Fehring (2005) stated that BBT is not completely accurate in predicting ovulation. An increase in BBT along with the occurrence of ovulation was only 22.1% accurate, while urinary LH concentration or OES:PRO ratios were approximately 93% accurate. Previous authors report the magnitude of change in BBT is greatest between the hours of 0300h-0500h (Hessemer & Bruck, 1985), and elicit a circadian rhythm (Cagnacci et al., 2002). The peak amplitude can easily be missed with only a single measurement. It was not logistically feasible to have participants in the laboratory at such hours, and scheduling restraints caused the trials to take place at various times of the day between subjects. Within individual subjects however, trials took place no more than 1.5 hours difference between, and therefore circadian influences were not likely the cause. So while an increase in BBT seems to be characteristic for menstrual cycle research, it is neither a consistent nor entirely reliable finding.

The subsequent major findings of this study were inconsistent thermoeffector threshold and gain alterations. Only ΔT_{resw}, and T_{resw}, were elevated during trial 2, with a trend for increased sweating gain. These findings agree with others (Gonzalez et al., 2002; Hessemer & Bruck, 1985; Kolka & Stephenson, 1989), yet contradict those reporting an elevated shivering threshold, and decreased shivering gain (Hessemer & Bruck, 1985; Grucza et al., 1997; Gonzalelz & Blanchard, 1989). Concomitant shifts in BBT and both thermoeffector threshold temperatures indicated an altered central oscillator with changes in sex hormone concentrations (Stephenson & Kolka, 1985). However, because shivering was not similarly affected, central control is unlikely modified. Instead, different mechanisms might occur during hot and cold stresses or in previous literature, the mechanism for a modified cold response was actually peripheral. A peripheral cold mechanism is probable with findings such that cutaneous thermal conductance decreased in the LP during 90 minute exposure to 28°C air (Frascarolo et al. 1990). Heat transfer to the periphery was minimized, and insulation provided by the shell increased. Skin sensitivity to the cold was enhanced in the LP and during PRO administration at the beginning of the cycle to mimic ovulation

(Kenshalo, 1966). Even though the cold effectors are innervated by efferent messages received from the central nervous system, the efferents and perception of thermal stimuli first depend on afferents from peripheral thermoreceptors (Saladin, 2001). Fluctuating PRO and OES content may directly influence depolarization of peripheral cold-sensitive receptors, although difficult to determine. Early animal studies indicated thermal information responding to cold stimulus was delivered only from receptors in the periphery (Simon, 1972). Few are located deeper in the body, unlike those that are warm-sensitive. Furthermore, a study conducted on female monkeys throughout the estrous cycle found smooth muscle that was transplanted into the primates constricted to different diameters, independent of nervous system communication but dependent on hormonal content ([Nafe, 1934], as referenced by Kenshalo, 1966). It is evident that while a greater concentration of PRO is in circulation, neuronal stimulation of the peripheral cold receptors is altered, and cold effector activity is affected in return. Shell variability was minimized in the present protocol to remove the variability of afferents from the cold thermoreceptors. Given that cold receptors are located predominantly in the periphery and significant phase differences were not found after removal of their influence, the mechanism responsible for menstrual phase adjustments during cold stress is superficial.

On the other hand, given that sweating thresholds were different and the peripheral drive was essentially equal between phases, the menstrual cycle alterations during heat stress are likely central in origin. Previous literature found that greater PRO concentration decreased stimulation of the warm-sensitive neurons in rats (Nakayama et al., 1975). It is conceivable that with increased PRO, depolarization of warm-sensitive neurons in the hypothalamus is attenuated or perturbed, and interrupts the efferents responsible for sweat gland stimulation. After all, neuronal depolarization is dependent not only on the synapses it directly receives; neurons located elsewhere in the nervous system, and the sensitivity of the neurons that integrate afferent information also affect the delivery of efferent transduction (Simon, 2000). Complicated feedback loops are created between the numbers of PRO receptor-complexes formed in target cells, and the thermoregulatory control of the hypothalamus (Thagard, 2002). Furthermore, thermoreceptors located in the spinal cords of rats and felines responded

only to heating, and minimally to cooling. Meanwhile, those located in the periphery were less influenced by warming (Simon, 1972). This shows that the drive for heat loss is dominated by a central component, thus subject to hormonal influence in this experiment. Of course this mechanism cannot be assured as findings require extrapolation from animal populations while no human data exist. Nonetheless, since a central component is more influential on heat loss responses and central drive was emphasized in the present protocol, the sweating threshold changed significantly. The varying concentrations of PRO modify the sweating threshold and gain because the hormonal adjustment during heat stress and physiological need for sweating are both centrally occurring.

Only one other study to our knowledge has examined the same individuals in variable thermal stress throughout the menstrual cycle. This classic study by Hessemer & Bruck (1985) was pivotal in justifying the altered set-point hypothesis because hot and cold responses were affected equally. Below in *Table 6.1.*, data is illustrated from each to compare thermal responses between studies in each trials.

Study	Variable	Significance
Dies (unpublished)	**Basal body temperature**	**NS**
Hessemer & Bruck (1985)		↑ $P < 0.0001$
Dies (unpublished)	**Basal metabolic rate**	**NS** (↑, $P = 0.066$)
Hessemer & Bruck (1985)		↑ $P < 0.01$
Dies (unpublished)	**Sweating cessation core temperature threshold**	↑ $P < 0.05$
Hessemer & Bruck (1985)		↑ $P < 0.01$
Dies (unpublished)	**Shivering onset core temperature threshold**	**NS**
Hessemer & Bruck (1985)		↑ $P < 0.01$
Dies (unpublished)	**Sweating gain**	**NS** (↑, $P = 0.10$)
Hessemer & Bruck (1985)		↑ $P < 0.01$
Dies (unpublished)	**Shivering gain**	**NS**
Hessemer & Bruck (1985)		**NS** (↑, $P > 0.10$)

Table 6.1. A comparison of dependent variables in the only two studies examining the same participants in both heat and cold stress throughout the menstrual cycle. (NS = not significant; ↑ = increased).

As evident in the table, there are controversies between studies. They likely result from methodology. In Hessemer & Bruck (1985) hot air was increased from 18-58°C for 12-15 minutes until sweating, and cold air was ramped from 18°C at -1°C•min^{-1} until shivering. From these hot and cold tests, more variables were found different between phases, than in the present experiment. Although more significant phase differences in the other study may stem from disparate testing schedules, they could be consequences of extreme air temperatures, and more mild changes in T_c. Below is another table (*Table 6.2.*) to compare the same studies, however examine ΔT_c at the thermoeffector thresholds. (Note: the effector responses were quantified very similar—shivering with $\dot{V}O_2$, and sweating by calculating change in water vapour over a local site).

Study	Phase	Response	ΔT_{re} (°C)	ΔT_{ty} (°C)
Dies (unpublished)	**Follicular**	**Sweating**	0.27	0.58
Hessemer & Bruck (1985)			0.01	0.03
Dies (unpublished)	**Follicular**	**Shivering**	0.38	0.57
Hessemer & Bruck (1985)			0.06	0.21
Dies (unpublished)	**Luteal**	**Sweating**	0.50	0.74
Hessemer & Bruck (1985)			0.10	0.23
Dies (unpublished)	**Luteal**	**Shivering**	0.30	1.20
Hessemer & Bruck (1985)			0.10	0.08

Table 6.2. A comparison of relative changes in rectal (T_{re}) and tympanic (T_{ty}) core temperatures following varied methods of heating and cooling to the participants in both heat and cold stress throughout the menstrual cycle.

In Hessemer & Bruck (1985), the ambient temperatures were severe, and delivered in a dynamic fashion. Exposure time was less, and disallowed T_c to change drastically before thermoeffector activation. Therefore, the drive for sweating and shivering was most likely from $\overline{T}_{sk}$ changing so rapidly, and thermal afferents were delivered from peripheral receptors only. In comparison, the rationale for utilizing stable water immersion was to isolate the central drive, remove dynamic changes in $\overline{T}_{sk}$, and provide a gradual, uniform thermal stress to the core. This experiment established that the cold response drive—proven to be more peripheral—is altered by fluctuating hormones when central thermoregulation is not targeted. Conversely, the heat response drive—which is more central—is altered by different hormonal concentrations whilst central thermal control is isolated. This experiment verifies that fluctuating PRO and OES affect thermoeffector activity by modulating only the component driving the thermoregulatory response. The contribution of the periphery and core is different for heat production and heat loss, especially when ΔT_c is larger than 0.15°C (Stephenson & Kolka, 1989). As a result, the sex hormones adjust thermal response through temperature-distinct means. So far, although the lack of a biphasic BBT

pattern suggests that female research does not need to be menstrual cycle specific, the latter discussion states otherwise. The thresholds and intensities of thermal responses are altered despite a deficient BBT increase post-ovulation.

Provided that the T_{resw}, and ΔT_{resw}, were greater in trial 2, the width of the rectal ITZ was also influenced by menstrual phase. However, the resultant ITZ was skewed, wherein only the heat loss threshold widened the zone. Although it was not a primary goal of the study, these findings confirm the presence of an ITZ—T_c range which neither sweating nor shivering occur—and contradict the set-point theory (Hammel et al., 1968). Therefore, it is difficult to follow suit with the shifted set-point hypothesis when a reference temperature was not evident. In this study, the minimum ITZ width was 0.63°C (T_{re}) (*Table 5.4 A-B)*. A 0.63°C change is far greater than the magnitude corresponding with a set-point. When Cabanac & Massonet (1977) tested the set-point theory, they found oesophageal temperature sweating and shivering thresholds were separated by a mere 0.05°C. Again, it was not the purpose of this experiment to support or negate a particular thermoregulatory model; however, the absence of a reference temperature does not support the most accepted hypothesis that the set-point temperature is elevated during trial 2. Furthermore, future research should calculate heat storage across the menstrual cycle as an alternative hypothesis. Rather than using temperature as the regulated variable, heat balance can be monitored to determine a critical temperature versus a critical heat balance that the menstrual cycle may alter.

Other interesting findings were the differences between the HCG and NCG. The significant age discrepancy is not surprising. This reflects the prevalence of younger females who choose to utilize HCs based on lifestyle choices and marital status. The HCG was recruited based on the monophasic contraceptive. The steady hormonal dose was to serve these participants as controls. They were tested at two different times during the active dose. Unlike previous studies that examine contraceptive users during menses (placebo), those in this study were observed in the week following menstruation. Stable PRO and OES concentrations were hypothesized to eradicate alterations between phases, but demonstrate differences between groups. Irrespective of trial, the HCG illustrated a decreased tympanic sweating threshold, and a narrower ITZ width. This suggests that thermal sweating is enhanced with contraceptive

use. The presence of exogenous progestin may increase sweat sensitivity during heating. The natural and synthetic forms differ by their chemical structure and potency, making the endogenous hormone less powerful (Ferin et al, 1993). SP consists of a compound that is similar to testosterone, thus its effects are androgenic (Ferin et al., 1993). Given that men have greater and earlier sudomotor output (Bittel & Henane, 1975), this could explain the decreased T_{tysw}, in the HCG. In addition, thermal gains were not significantly different between groups, but T_{re} cooling rate was significantly faster in the HCG. As a result, the votes on subjective thermal scales were closer to "extremely uncomfortable," and "unbearably cold." It is well known that T_c cooling rate is highly dependent on body mass and subcutaneous tissue adiposity (Anderson, 1999). But as seen in *Table 5.1*, group demographics were not different, and could not account for a faster cooling rate. Unfortunately, few have examined cold responses in females utilizing HCs. A study by Grucza et al. (1997) exposed contraceptive users to cold air (3.4°C) for 30 minutes. T_{re} cooling rate was similar between phases, and near the same rate in the present study, approximately $1.10°C•hr^{-1}$. In comparison, the T_{re} cooling rate of non-HC users in a study by Tikuisis et al. (2000) was only $0.45°C•hr^{-1}$ while immersed in 18°C water. Perhaps synthetic hormones enhance heat extraction or exaggerate autonomic response during temperature stress. Participants in this experiment also elicited a substantially greater blood flow gain. This further indicates a more intense reaction to preserve heat. Taken together, the perception, and physiology of heat and cold extremes are more stressful in females that use HCs; sweating occurs earlier, vascular changes are greater, core cooling is quicker, and subjective comfort is less bearable while cooling. Unfortunately thermal response is not yet thoroughly examined in HCG users, leaving little research to contrast these females to non-user counterparts.

Another unexpected finding in the present study was significantly different basal and threshold temperatures between the tympanic and rectal sites. Oesophageal temperature would have provided a third and potentially more accurate representation of T_c (Moran & Mendal, 2000). However due to limitations, only T_{re} and T_{ty} were used. The following describes possible reasons for disparate measurements, as reviewed in detail by Moran & Mendal (2000). The tympanic membrane is contiguous with the vessels supplying the carotid artery, and ultimately the hypothalamus. Although seemingly flawless, dirt, insertion

depth, rapid head movement, or examiner skill can affect the accuracy of this measure. The experimenters described the insertion procedure equally to each participant, and gave best efforts to not reposition the thermometer when securing it. Despite this care, T_{ty} measurements were consistently lower than those of T_{re}. This finding however, is not uncommon according to the review article, and others (Bittel & Henane, 1975). T_{re} is considered most practical, produces an "average" core temperature, but is usually higher than other sites due to the proximity of viscera. Unfortunately, insertion depth can influence its accuracy, and because the thermal inertia of surrounding tissue is large, the response time lags. This delay was evident by the unanticipated direction of the sweating gain slope when plotted against ΔT_{re}. Overall, future investigation and comparisons should avoid using T_{ty} when T_{re} is available. Even still, oesophageal temperature is preferred due to a quicker response, and proximity to the blood leaving the aorta (Moran & Mendal, 2002). If between-study comparisons are being made, authors must evaluate the same anatomical location because of variability within the sites.

Chapter 7- Conclusions

1) Conclusions

Within the limits of the present experiment, it is concluded that:

1. An increase in basal body temperature during the luteal phase may not always be a marked physiological response in women, nor serve as an accurate indicator of ovulation consistently.
2. Quantifying thermoeffector thresholds with both tympanic and rectal temperatures provides remarkably different values within one trial. As a consequence, caution must be used when comparing core temperature between studies, and avoid using tympanic temperature alone, as results will be inaccurately low.
3. The central sweating threshold increases during the latter half of the menstrual cycle for both non-users and users of hormonal contraceptives. Between these populations however, hormonal contraceptive users display an overall decrease in the sweating threshold, irrespective of hormonal dose phase.
4. Sweating gain substantially increases during the latter half of the menstrual cycle for both non-users and users of hormonal contraceptives. However, shivering gain is unchanged throughout for either group.
5. The width of the interthreshold zone increases during the latter half of the menstrual cycle, due to an increase in the heat loss thermoeffector threshold. In addition, a decrease in the sweat threshold in hormonal contraceptive users decreases the width of the tympanic temperature interthreshold zone regardless of hormonal dose phase..

2) The direction for future research

The present study isolated central thermoregulatory control to establish the role of female sex hormones on central thermoeffector threshold temperatures, and width of the ITZ. Data shows the

extent of alterations depends on whether the drive for heat loss or production is central or peripheral. An interesting study can involve using this same protocol, but examine females with menstrual cycle disorders, such as amenorrhea or luteal phase deficiency (not enough circulating PRO). Thermal response may remain unchanged between phases when endogenous hormone concentrations are abnormally low, and support a critical level of PRO is required for thermal modifications. A longitudinal study that investigates females throughout the menstrual cycle before, and then 3 months after administering a HC, would also broaden understanding. Testing participants with a standardized protocol before and after HC use will better indicate if thermal sensitivity changes with exogenous hormones. Furthermore, to verify an increase in BBT during the LP, T_c can be monitored constantly using telemetry pills or continuous T_{re} measurement. Temperature can be recorded for 24 hours, every day throughout consecutive menstrual cycles for more vivid understanding. Even more so, to better understand the mechanisms of this potential shift in basal temperature, plasma cytokines may be quantified daily for consecutive cycles to prove or disprove the hypothesis of the LP fever-like state (Cannon & Dinarello, 1985). An alternative study could quantify an ambient temperature that is "preferred" for the whole-body pre- versus post-ovulation. This may determine whether peripheral thermal conductance is altered, and the peripheral mechanism hypothesized to alter BBT (Frascarolo et al., 1990). The effects of the menstrual cycle on overall heat storage would also be valuable to investigate. Finally, if sweating is induced locally via a pharmacological substance as opposed to whole-body heating—such as pilocarpine—whether PRO influences only the central drive for heat loss versus the peripheral stimulation of the sweat gland can be verified.

More generally speaking, future research should standardize T_c with the use of liquid conditioning garments or water immersion before exposure to thermal stress. Entering a given environment with the same T_c in both menstrual phases will provide a better understanding of hormonal influences on the peripheral organs involved in thermoregulation. Likewise, skin temperature can be clamped, like in this experiment, to improve understanding of the effects on central thermoregulation. Targeting either the core or shell helps to define the mechanisms of the female sex hormones on each of these

thermoregulatory components. Ideally the responses to cold and heat challenges should be investigated in the same participant to reduce inter-individuality, and provide more data to compare with the present experiment and Hessemer & Bruck (1985). As well, studies that involve both cold and heat challenges will better support or negate an altered central controller with increasing PRO. In participants not utilizing hormonal contraceptives, the occurrence of ovulation should coincide with a positive luteinizing hormone test or quantification of sex hormones, as BBT is not sufficient according to the present data. Finally, the effects of hormonal contraceptive use have yielded controversial data, and participants utilizing them should be examined as frequently as non-users, due to their prevalence in healthily ovulating, and amenorrheic females.

3) Limitations

This study was predicated on the hypothesis that thermoregulation occurs through the "reciprocal inhibition" temperature model. Another temperature model is the "set-point." In both these models, temperature is the regulated variable. In other words, the temperature value(s) which the body is sensed to be at will dictate the activation and intensity of the thermal effectors. However, there is another model called the "heat storage" model, wherein temperature is not the controlled variable. Instead, thermoregulation is achieved by sensing the amount of heat storage, and balancing heat gain with heat loss. Heat storage is not calculated with varying temperatures, but with dry and wet heat loss terms found in the heat balance equation. Consequently, future analysis should take into consideration the third and final human thermoregulatory model to further clarify the role of female sex hormones on thermoregulation.

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Appendices

Glossary of Important Terms

17β-OESTRADIOL[2]: A natural estrogenic hormone that is a phenolic alcohol of C18H24O2 secreted chiefly by the ovaries, is the most potent of the naturally occurring oestrogens, and is administered in its natural or semisynthetic esterified form especially to treat menopausal symptoms.

CRITICAL TEMPERATURE, LOWER[1]*:* The ambient temperature below which the rate of metabolic heat production of a resting thermoregulating animal must be increased by shivering and/or nonshivering thermogenesis in order to maintain thermal balance.

CRITICAL TEMPERATURE, UPPER[1]: The ambient temperature above which the rate of evaporative heat loss of a resting thermoregulating animal must be increased (e.g., by thermal tachypnea or by thermal sweating) in order to maintain thermal balance.

PROGESTERONE[2]: A female steroid sex hormone $C_{21}H_{30}O_2$ that is secreted by the corpus luteum to prepare the endometrium for implantation and later by the placenta during pregnancy to prevent rejection of the developing embryo or fetus; also **:** a synthetic steroid resembling progesterone in action.

THERMAL NEUTRAL ZONE (TNZ) [1]: The range of ambient temperature at which temperature regulation is achieved only by control of sensible heat loss, i.e., without regulatory changes in metabolic heat production or evaporative heat loss.

THERMOEFFECTOR[1]: An organ system and its action, respectively, that affect heat balance in a *controlled* manner as part of the processes of temperature regulation.

THERMOEFFECTOR GAIN[1]: The derivative of the thermoeffector output with respect to body temperature deviation from the set-point.

THERMOEFFECTOR THRESHOLD[1]: The level of activity of a potential thermoeffector that is transgressed when it becomes actively involved in temperature regulation. The thresholds of other thermoeffectors are arbitrarily defined, or agreed upon by convention, because basal or resting levels are difficult to define, e.g., in case of circulatory convection of heat to the skin and the underlying cutaneous vasomotor tone.

THERMOEFFECTOR THRESHOLD TEMPERATURE[1]: Describes the level of a specified body temperature (e.g., core temperature or mean body temperature) the transgression of which in one direction, either upward or downward, will activate a certain thermoeffector. As a rule, the threshold core temperature determined for a given effector will be a function of skin temperature, and vice versa.

THERMOEFFECTOR THRESHOLD ZONE (INTERTHRESHOLD ZONE) [1]: The temperature range between two threshold (body) temperatures, for activation of any thermoeffector responses, particularly of metabolic heat production and of evaporative heat loss when no thermal load is present. This special steady-state may be called set-point.

Definitions obtained from:

[1] IUPS Thermal Commission. (2001). Glossary of terms for thermal physiology, 3rd ed. *Japanese Journal of Physiology, 51*, 245-280.

[2]Merriam-Webster's Medical Desk Dictionary. (2005). Retrieved Jun 5, 2009, from http://merriam-webster.com/medical.

Thermal Comfort Vote

1.	Comfortable
1.5	
2	Slightly uncomfortable
2.5	
3.	Uncomfortable
3.5	
4.	Very uncomfortable
4.5	
5.	Extremely uncomfortable

Thermal Sensation Scale

0	unbearably cold
1	very cold
2	cold
3	cool
4	slightly cool
5	neutral
6	slightly warm
7	warm
8	hot
9	very hot
10	unbearably hot

Informed Consent: EEL-053

Project Title: The effect of the menstrual cycle and oral contraceptive use on central thermoeffector threshold temperatures and the interthreshold zone width.

Principal Investigator:
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INVITATION

You are invited to participate in a study that involves research. The purpose of this study is to examine how natural and synthetic female sex hormones affect the core temperature shivering and sweating thresholds. Whether the oestrogens and progestogens that fluctuate throughout the menstrual cycle affect the central activation of thermal responses is unknown. As well, whether monophasic contraceptives alter when you shiver or sweat has yet to be determined. The shivering and sweating core temperature threshold measurements will give us information on how the hormones affect the thermoregulatory system, and may benefit females in many settings, including athletes or industrial employees.

WHAT'S INVOLVED

The first testing session will occur during the early follicular phase of the menstrual cycle (days 2-5), and the second testing session will occur during the late luteal phase of the menstrual cycle (4-8 days post-ovulation, or days 18-22 if you are prescribed a contraceptive). Time commitment will be approximately 8 hours over the two sessions, spaced out over one menstrual cycle. In addition, you will be asked to refrain from alcohol and/or heavy exercise for 24 hours prior to the trial, and caffeine on the day of the trial. You will be wearing a bathing suit for all the sessions, and will have access to change rooms for you to change into the required clothing.

In the first session, your height, weight, and amount of body fat will be measured. Body fat testing will be performed using skinfold calipers, which might cause a slight pinching sensation, and will be taken by someone of the same sex in a private room. You will receive a treatment of pilocarpine iontophoresis while seated. The drug is housed within two specially designed electrodes that are securely attached to your forearm. An electrical current flows through the electrodes to allow the drug to perfuse through your skin and stimulate the sweat glands under the area of the electrodes. The electrical current is of very low amperage, and may or may not provide a sensation similar to that of "pins and needles." Following 5 minutes of the drug delivery, specially designed sweat collection tubing will be attached to your forearm where the sweat was produced. Within 20 minutes, the sweat collection tubing will be removed and analyzed for the amount of sweat produced and its tonicity. After this seated measurement, you will perform 30 minutes of underwater exercise at an intensity of 50% of the estimated maximal heart rate based on your age. Depending on how much sweat is produced on your forehead, the exercise may last slightly less or longer than this estimated time. The exercise condition will involve pedaling on a bike immersed in water that is level to the underarms, and at a comfortable 28°C. Following this exercise, you will remain seated in the water, but only to rest. You will remain seated until you are shivering steadily. During both sessions, your internal temperature will be measured by wearing temperature sensors in the rectum and tympanic membrane. The rectal sensor consists of a very thin and flexible plastic tube that you

insert 15 cm beyond the anus. The tympanic sensor is a specially designed tube with a valve at the end, to keep the probe in place once you insert in your ear canal close to touching the membrane. Placed on your forehead will be a plastic sweat capsule that will collect the rate at which you are sweating at that site. Near the sweat capsule will be a laser Doppler probe that will determine the rate of blood flow at the forehead. You will have your heart rate monitored using a sensor in a flexible strap around the chest. Skin temperature and heat flow will be monitored using wires taped to various sites on your body. Your oxygen uptake will be measured by having you breathe through a soft silicone mask. You will also be asked at different times to give your subjective feeling of your effort and also your thermal sensation and comfort. A technician of the same sex will be available to assist with the dressing and instrumentation. The sessions will take about 3.5 hours. The first session will occur pre-ovulation, while the second will occur "post-ovulation," or during the high hormone dosage of females using a contraceptive.

POTENTIAL BENEFITS AND RISKS

Possible benefits of participation include your receiving a body composition that is a quantification of your lean body mass. The fitness test will help you understand your fitness level and the intensity that best suits you for general exercise training. This study will also provide information on how your menstrual cycle and the fluctuating estrogens and progestogens alter your body's autonomic ability to regulate temperature.

There also may be risks associated with participation. The nature of underwater exercise may leave your muscles feeling quite tired for up to 24 hours after the test and experimental sessions. There is a very remote risk of heart attack or stroke when exercising or cooling in the water, but this is minimized with the use of the health screening questionnaire. There will be at least two investigators trained in First Aid and CPR present for each experiment.

Experimental sessions will be terminated if:

1. Rectal temperature increases beyond 39.5°C.
2. Rectal temperature decreases beyond 36.0°C
3. Heart rate has risen above 95% of its maximum (220-age) for 3 min.
4. Dizziness or nausea precludes further experimentation.
5. Subject decides, for any reason, to end the experiment.
6. The investigators determine that the subject cannot tolerate the thermal stress in order to continue.

If you experience any unusual symptoms after completing a testing session, you should immediately seek medical attention and inform Dr. Cheung and Natalie Dies. The investigators will also contact you the evening of your participation to ensure that you are in a healthy state. Depending on your health status, you may be asked to consult with a physician.

Insertion of the flexible rectal probe may cause slight discomfort. You will be given instruction about how to prepare the probe, and will self-insert the probe in a private room. You will be provided with water-based lubricant if necessary, and will secure the probe with a soft gauze "sumo sling" harness which will keep it in place during exercise. Insertion of the flexible tympanic probe may cause slight discomfort and cause you to hear a constant humming noise. The probe is specially designed with a valve-like system to prevent the probe from perforating your tympanic membrane. You will insert it into the auditory canal until it touches the membrane. The probe is then withdrawn slightly to alleviate any initial discomfort. The probe and ear are then covered with a soft, circular foam pad that is gently secured to the head with a strap. There is a slight but real risk of perforations to the bowel from the insertion of the rectal probe, and the tympanic membrane from the insertion of the tympanic probe, though the investigators are unaware of these occurring in a research setting.

Some of the symptoms that may be experienced with a decreased body temperature include: discomfort, pain, fatigue, shivering, loss of fine motor coordination, minor mental confusion and irritability. These symptoms, except for fatigue like that from mild exercise, commonly disappear almost immediately upon return to normal body temperature. Some of the symptoms that may be experienced with an elevated body temperature include: discomfort, sweating, flushing and redness in the face and body, thirst, loss of fine motor coordination due to sweating, minor mental confusion, dizziness or nausea. These symptoms, except for thirst, commonly disappear almost immediately upon return to normal body temperature. You will be given as much fluid as desired at the end of the experiment. You will be asked to stay in the lab for 45-60 min following each experiment, and your core temperature, heart rate, blood pressure, and hydration status will be monitored.

RECTAL PROBES

When performed in a healthcare setting, insertion of the rectal probe is a controlled act as set out in the Regulated Health Professions ACT. While this act does not extend to research outside of a healthcare setting, you should be aware of the following potential risks:

- Insertion of the rectal probe can stimulate the vagus nerve which can cause slowing of the heart rate which may lead to fainting. This is more likely to happen if you have a low resting heart rate.
- Perforation of the bowel can lead to peritonitis, a serious infection of the abdominal cavity.
- You should not participate in this research if you are pregnant, diabetic, have a history of esophageal problems (including reflux esophagitis), are under the influence of alcohol or other sedating substances (tranquilizers, sleeping pills, street drugs) or have any history of fainting or heart disease.

CONFIDENTIALITY

Access to this data will be restricted to Dr. Cheung, Ms. Natalie Dies, Mr. Raffy Dotan (research technician), Mr. Shane Kilburn, Mr. Geoff Hartley, Mr. Matthew Smith, Ms. Nikki Zouros, Ms. Leah Rosso (research assistants). Your participation will remain confidential. The data collected from this investigation will be kept secured on the premises of the Department of Physical Education and Kinesiology (PEKN) at Brock University in Dr. Cheung's office or laboratory, and will not be accessed by anyone other than the listed investigators. The data (paper and electronic) will be destroyed five years after the publication of the results of the study.

Investigators will require disclosure of your name and contact information (phone, email), and therefore your participation is not anonymous during the conduct of the research. All participants will have their names removed from any data. The master list matching participants to data will be kept by Natalie Dies, and will be destroyed following the publication of data. All information you provide is considered confidential; your name will not be included or, in any other way, associated with the data collected in the study. Furthermore, because our interest is in the average responses of the entire group of participants, you will not be identified individually in any way in written reports of this research.

VOLUNTARY PARTICIPATION

Participation in this study is voluntary. If you wish, you may decline to answer any questions or participate in any component of the study. Further, you may decide to withdraw from this study at any time and may do so without any penalty or loss of benefits to which you are entitled. Participation, non-participation, or withdrawal from the study will not affect your standing at Brock University.

PUBLICATION OF RESULTS

Results of this study may be published in professional journals and presented at conferences, but your personal information and participation will remain confidential. Approximately one month after we finish testing all participants, we will provide you with a summary of your own results and also the overall group results. Feedback about this study will be available from Natalie Dies (nat.dies@brocku.ca, 289-668-0771).

CONTACT INFORMATION AND ETHICS CLEARANCE

If you have any questions about this study or require further information, please contact the Principal Investigator using the contact information provided above. This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock University REB 08-058. If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

CONSENT FORM

I agree to participate in this study described above. I have made this decision based on the information I have read in the Information-Consent Letter. I have had the opportunity to receive any additional details I wanted about the study and understand that I may ask questions in the future. I understand that I may withdraw this consent at any time. My participation, non-participation, or withdrawal from the study will not affect my standing at Brock University.

Name: _____

Signature: _____ Date: _____

Thank you for your assistance in this project. Please keep a copy of this form for your records.

The effects of the menstrual cycle and hormonal contraceptive use on the thermoeffector threshold temperatures and width of the interthreshold zone (EEL 053)

Environmental Ergonomics Laboratory Fitness Screening Form

Please read over the questions below*. They are to assist in assessing whether you are fit to participate in this study. Please ask the investigators if you have any queries while filling out the form.

Screening Questions*	YES	NO
1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?		
2. Do you feel pain in your chest when you do physical activity?		
3. In the past month, have you had chest pain when you were not doing physical activity?		
4. Do you lose your balance because of dizziness or do you ever lose consciousness?		
5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?		
6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?		
7. Do you know of any other reason why you should not do physical activity?		
8. Do you have diabetes?		
9. Do you have any bowel or prostate problems (e.g. colitis, irritable bowel syndrome, prostate problems)		
10. Neuromuscular (e.g., epilepsy, Multiple Sclerosis, Cerebral Palsy) or skeletal (e.g., inflammatory or degenerative arthritis) disorders?		

Name: _____

Signature _____ Date** _____

** The wording for questions 1-7 are directly copied from the Physical Activity Readiness Questionnaire (PAR-Q) developed by the Canadian Society for Exercise Physiology (www.csep.ca). Please let the investigator(s) know if you would like to see a copy or for more information.*

*** This screening form becomes invalid if your condition changes so that you cannot answer "no" to any of the questions. Please immediately inform the investigators if this is the case or contact Dr. Stephen Cheung (905-688-5550x5662) if you require further information.*

EEL 053 Menstrual Cycle History Questionnaire

(1) When was your last menstrual period? Date: _____

(2) On average, how long does your menstrual period last (in days)? _____

(3) How would you rate your overall *discomfort* during your menstrual period? (Indicate with a check mark on the line scales below)

Uterine cramping: Very uncomfortable ----------Comfortable
Back pain: Very uncomfortable ----------Comfortable
Mood swings: Very uncomfortable ----------Comfortable
Change in appetite: Very uncomfortable ----------Comfortable
Ability to maintain a comfortable temperature without adding or removing clothing:
Very uncomfortable ----------Comfortable

(4) On average, how heavy would you rate your blood flow?: Light ----------Heavy

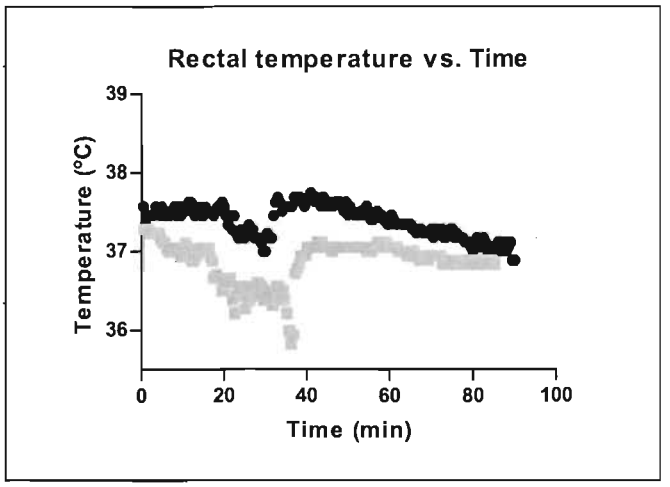
(5) Have you ever had a menstrual cycle lasting longer than 42 days (the time between two periods)?
YES ☐ NO ☐
If yes, when? _____

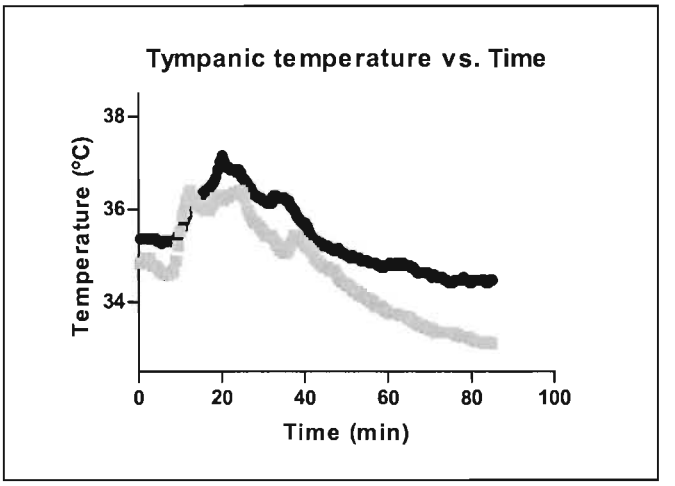
(6) Have you ever had a menstrual period with heavy blood loss, lasting longer than 7 days?
YES ☐ NO ☐
If yes, when? _____

(7) Have you ever had an absence of period for 6 months or longer?
YES ☐ NO ☐
If yes, when? _____

(8) Have you ever been pregnant, or are you currently?
YES ☐ NO ☐
If yes, when? _____

***NOTE**:* The following graphs were created prior to classifying the follicular phase (FP) as Trial 1, and the luteal phase (LP) as Trial 2 in the present experiment. Therefore, all data that displays **FP** data corresponds to **Trial 1**, and **LP** data to **Trial 2**.



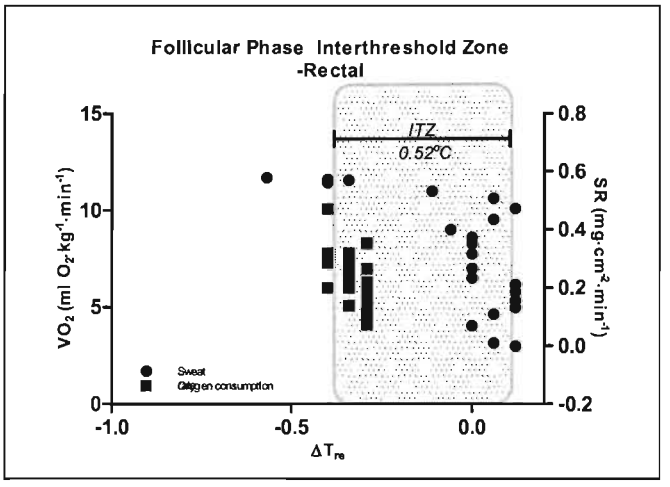


Subject *AS*

Fig 1. and **Fig 2.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



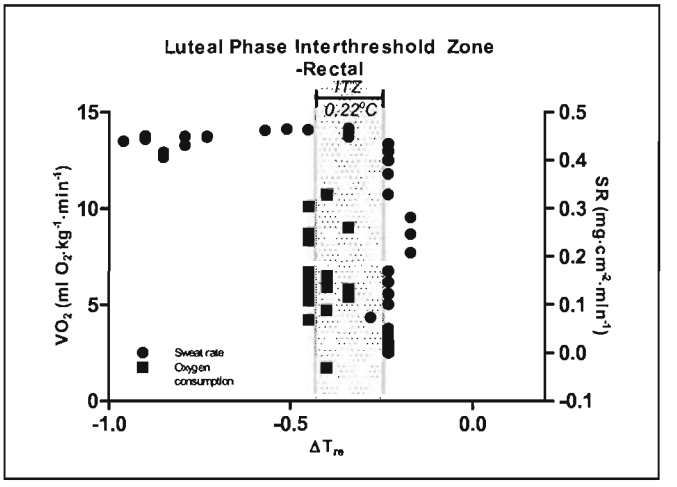
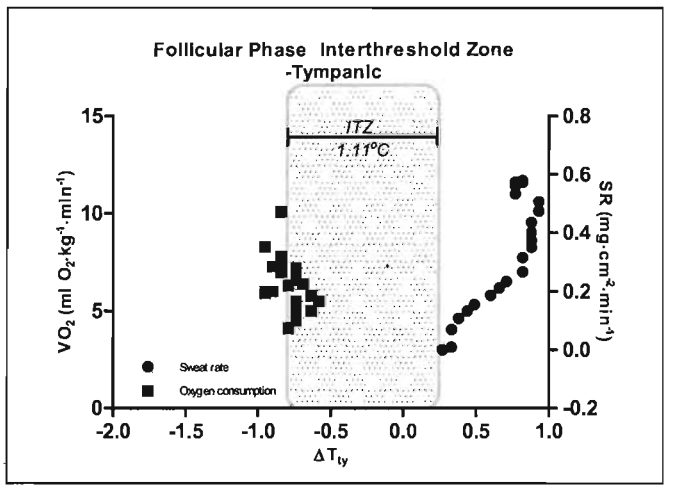
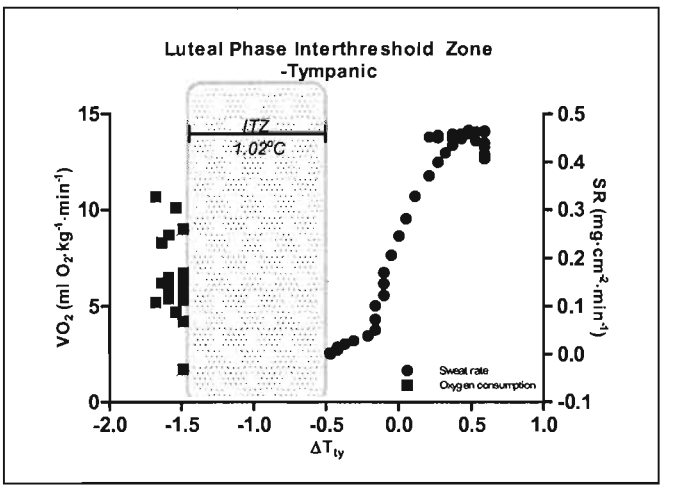
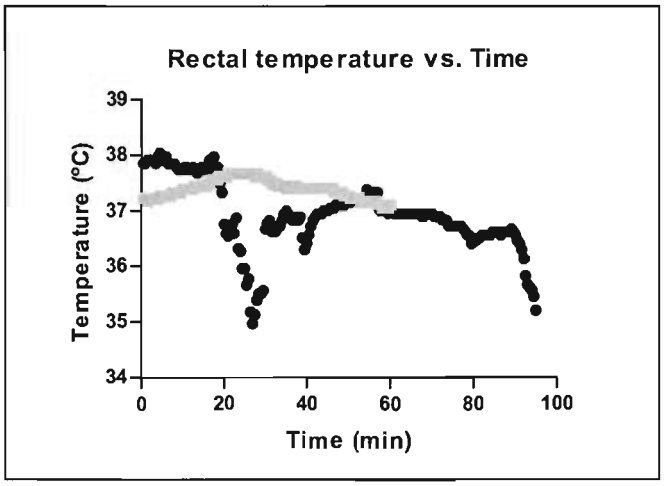


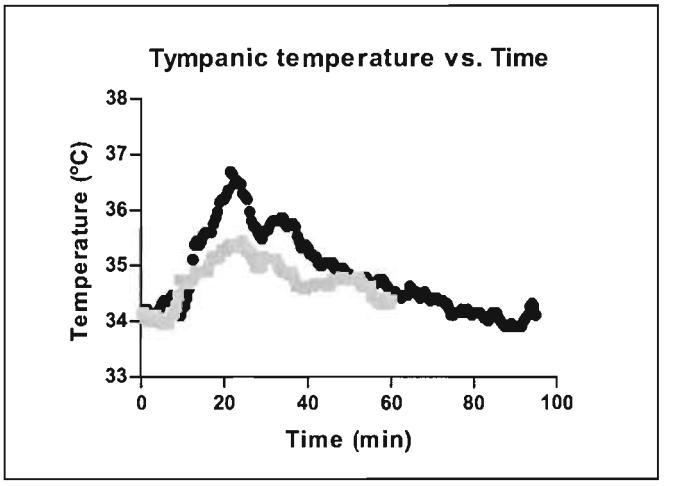
Fig 3. and **Fig 4.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 5. and **Fig 6.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







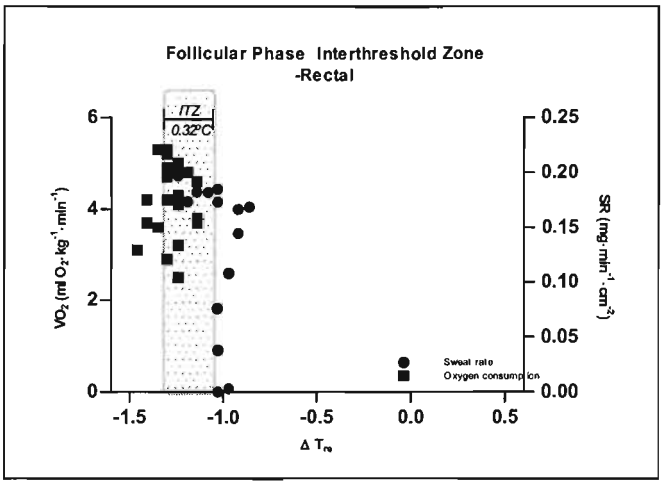


Subject *BD*

Fig 7. and **Fig 8.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



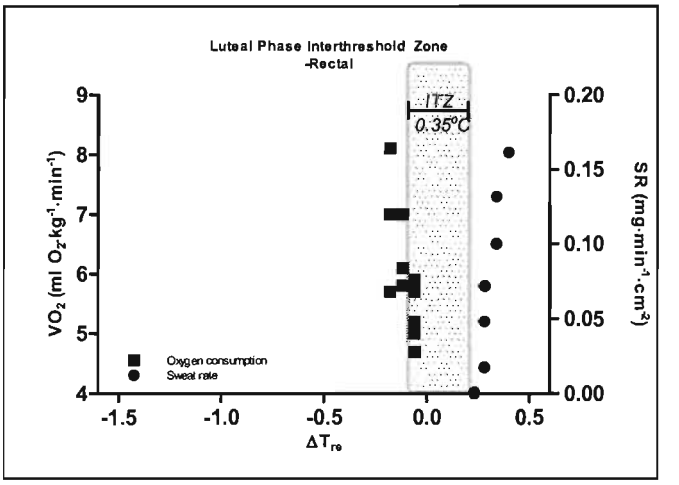
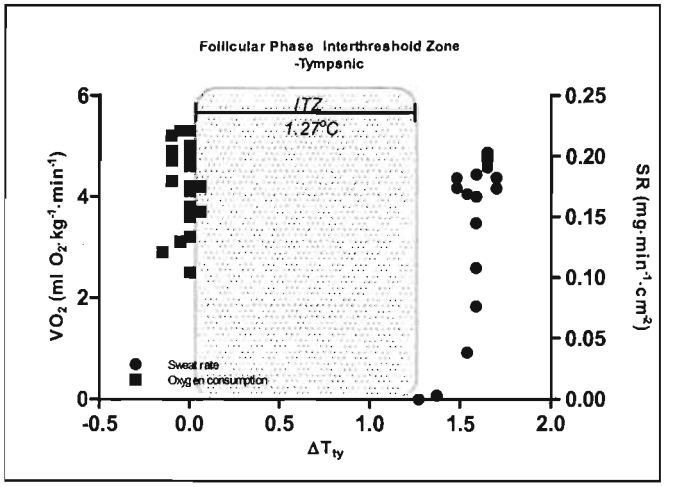
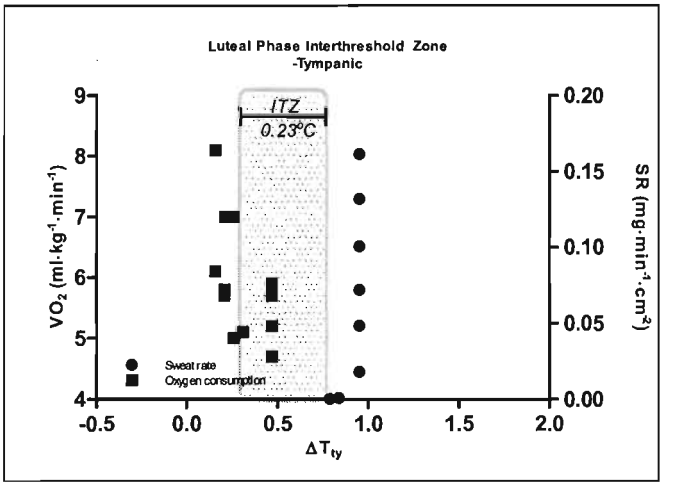
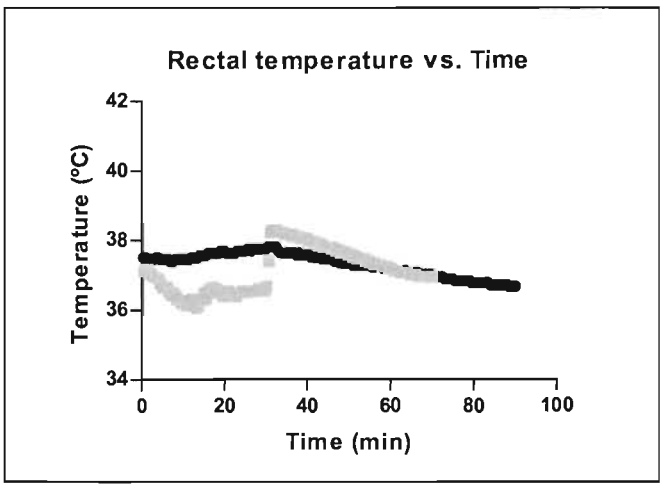


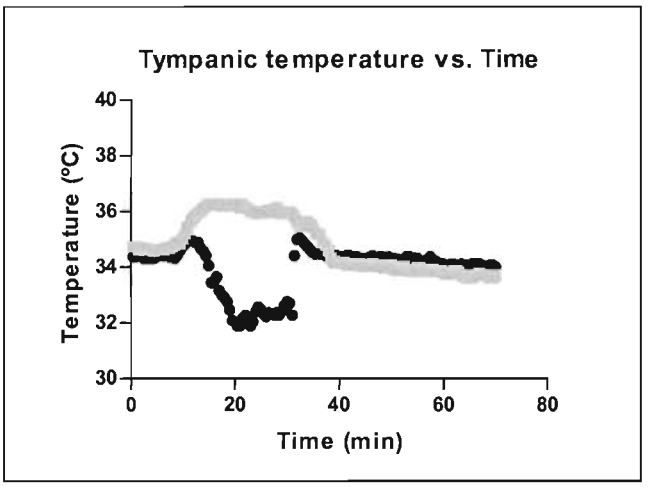
Fig 9. and **Fig 10.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 11. and **Fig 12.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







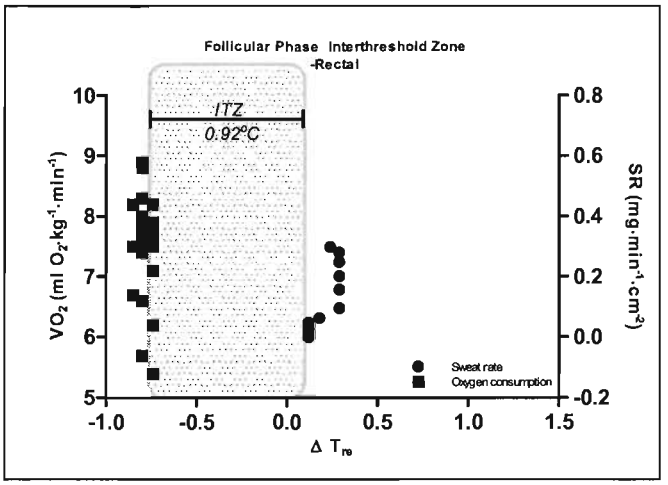


Subject *CG*

Fig 13. and **Fig 14.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



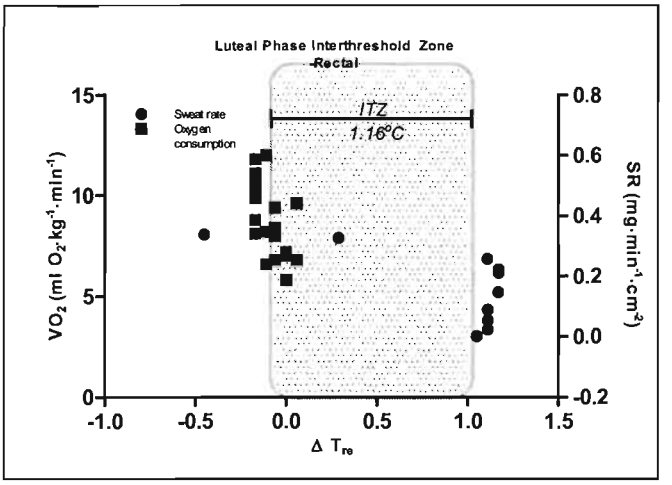
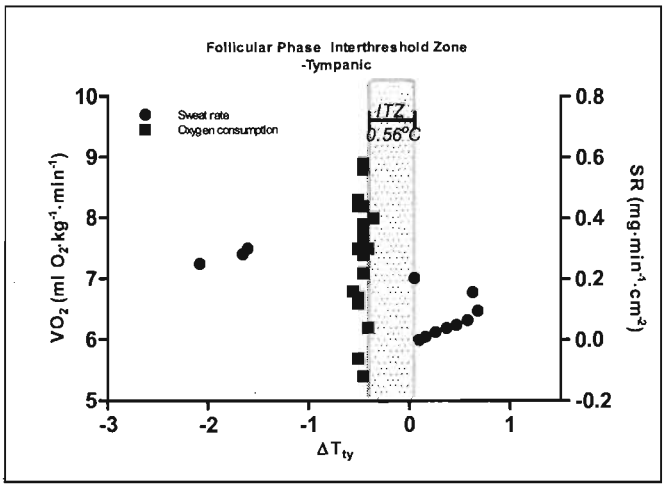
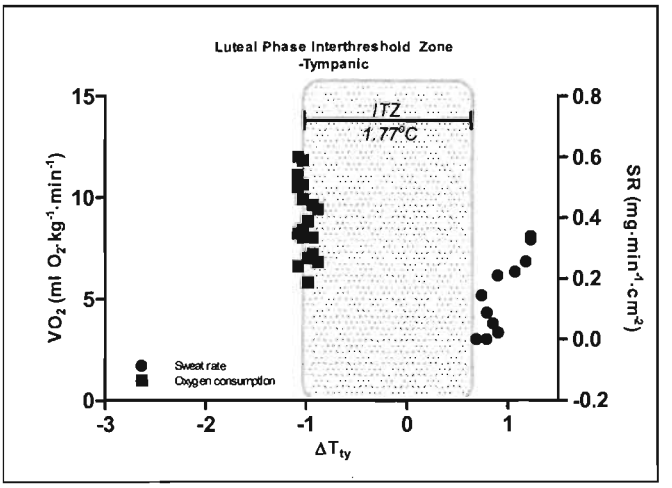
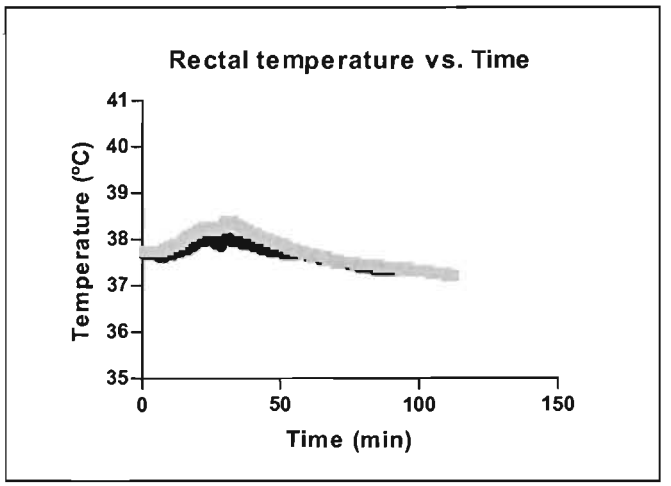


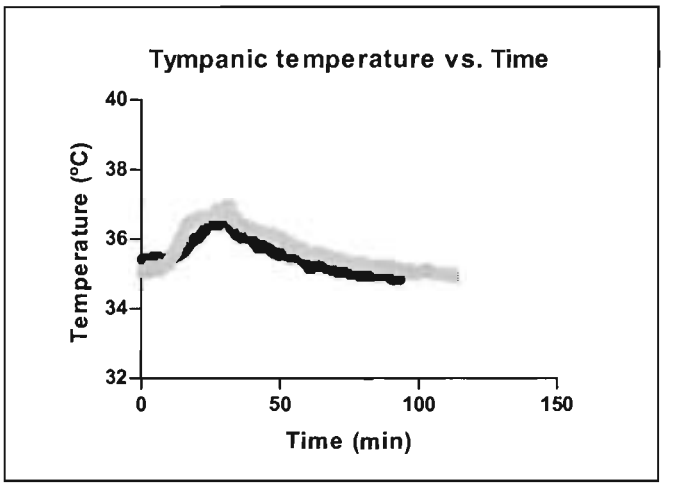
Fig 15. and **Fig 16.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 17. and **Fig 18.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







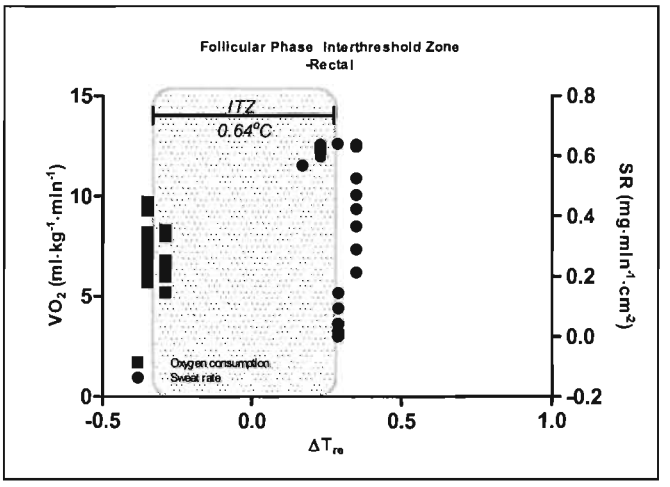


Subject *CM*

Fig 19. and **Fig 20.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



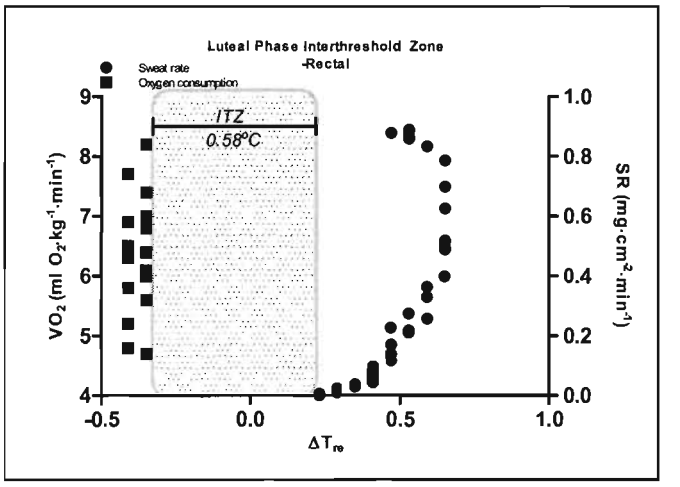
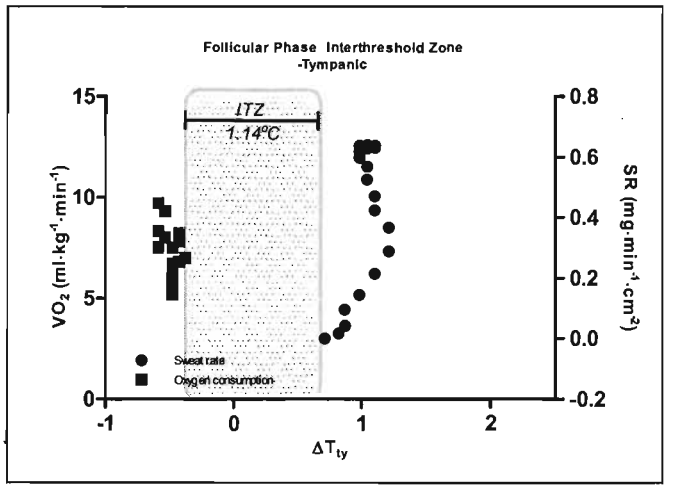
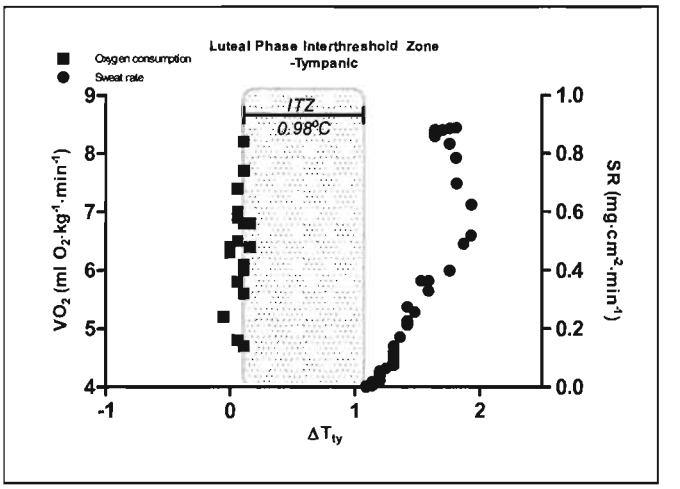
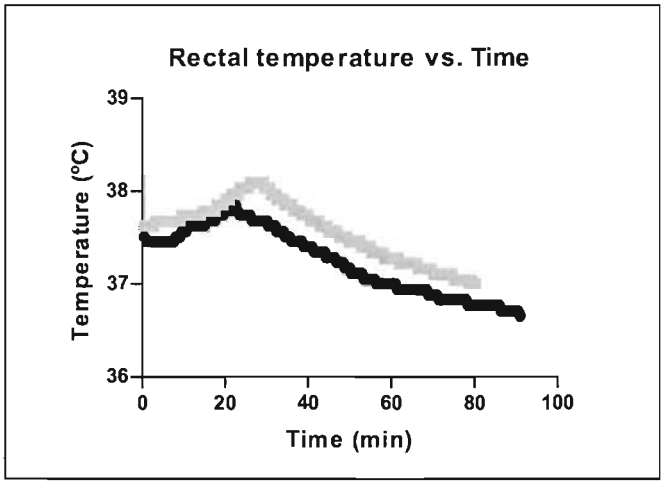


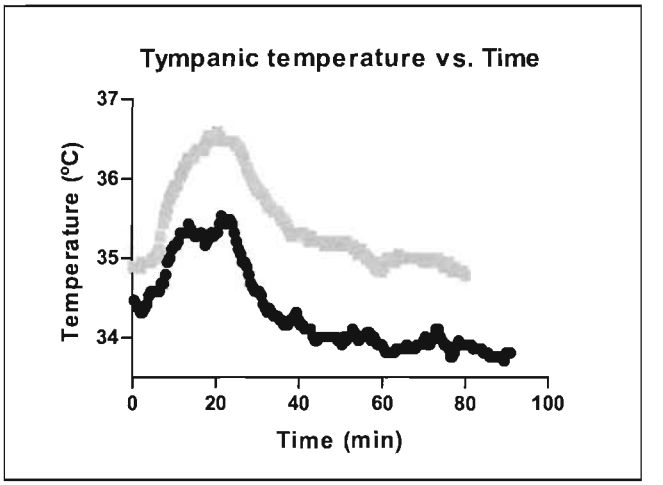
Fig 21. and **Fig 22.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 23. and **Fig 24.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







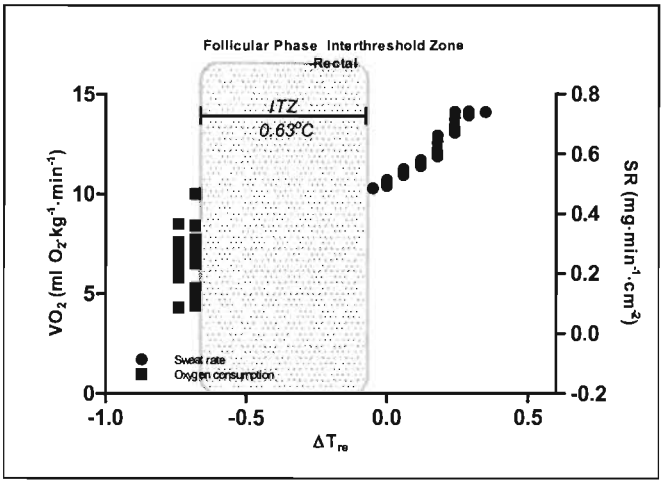


Subject *ED*

Fig 25. and **Fig 26.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



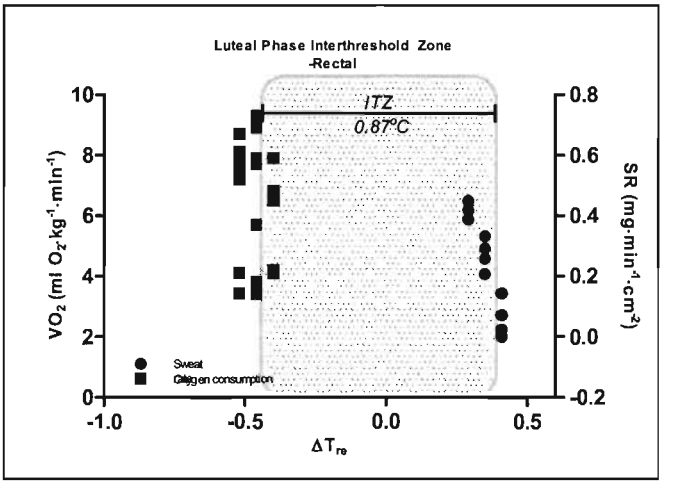
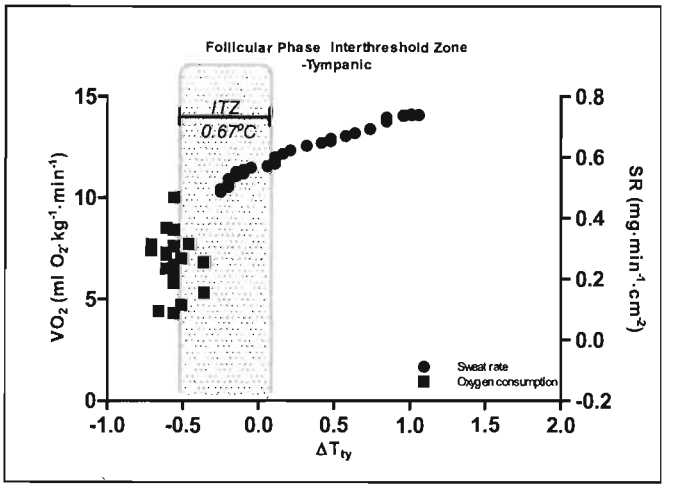
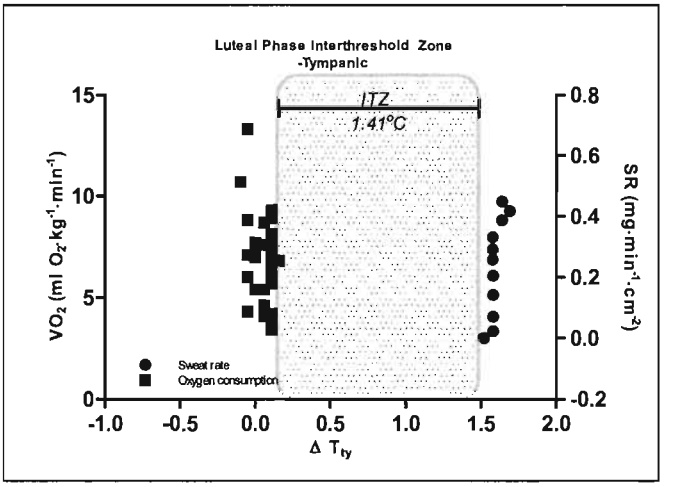
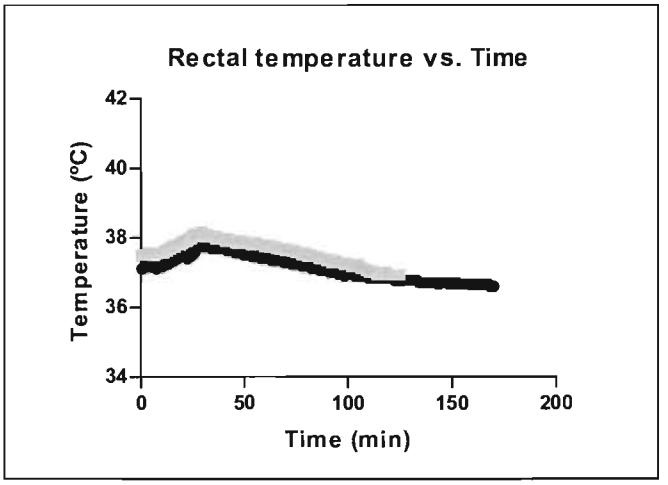


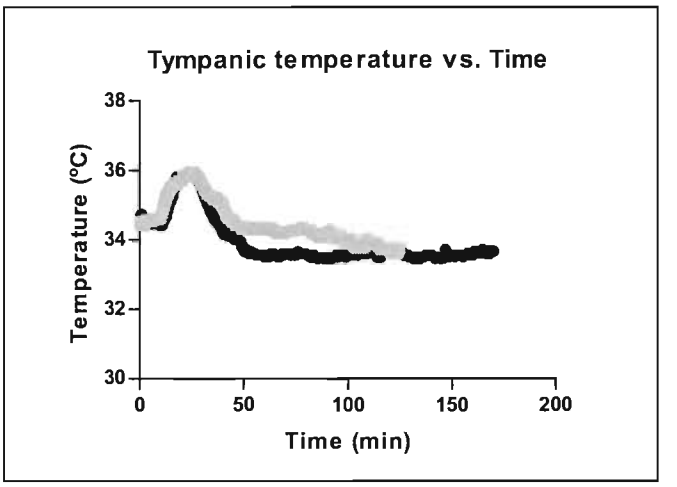
Fig 27. and **Fig 28.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 29. and **Fig 30.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







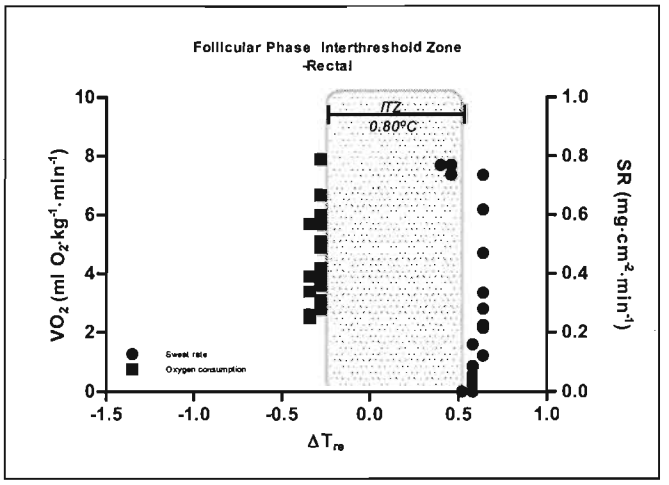


Subject *LC*

Fig 31. and **Fig 32.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



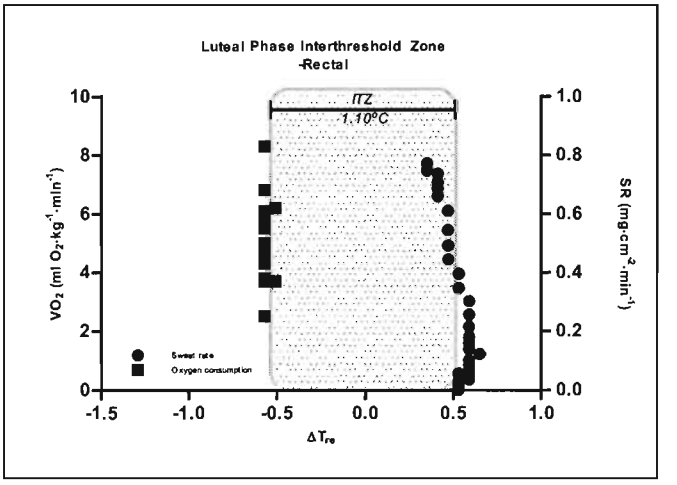
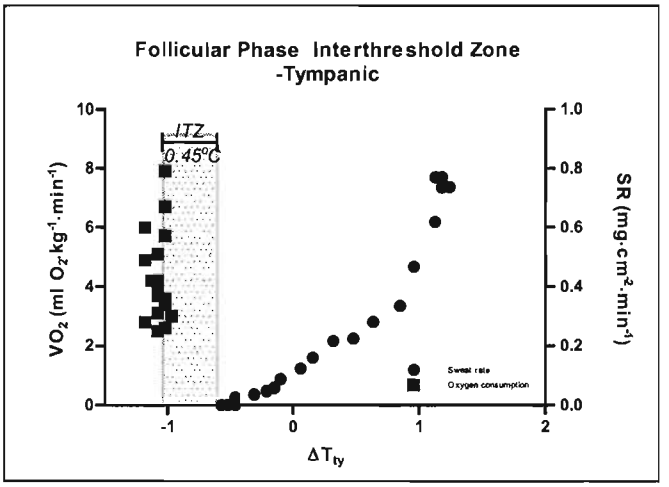
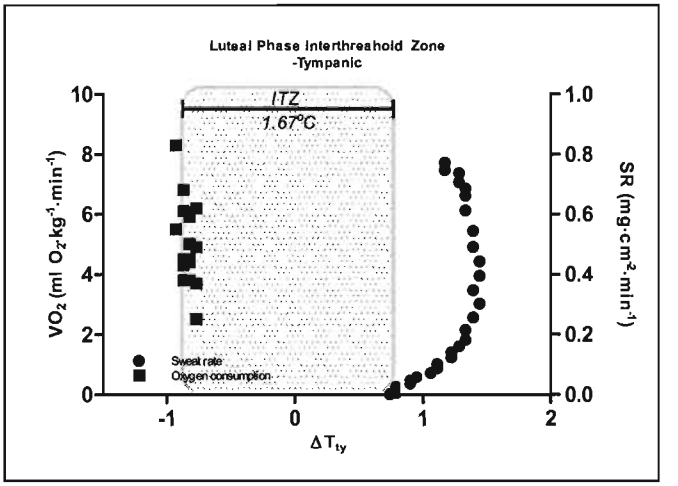
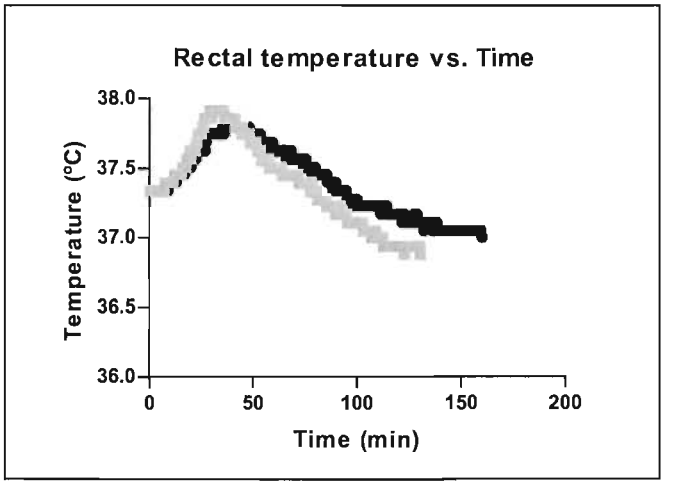


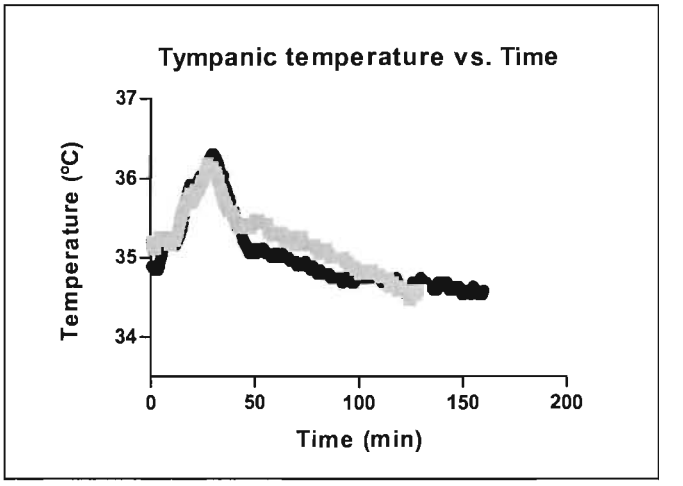
Fig 33. and **Fig 34.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 35. and **Fig 36.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







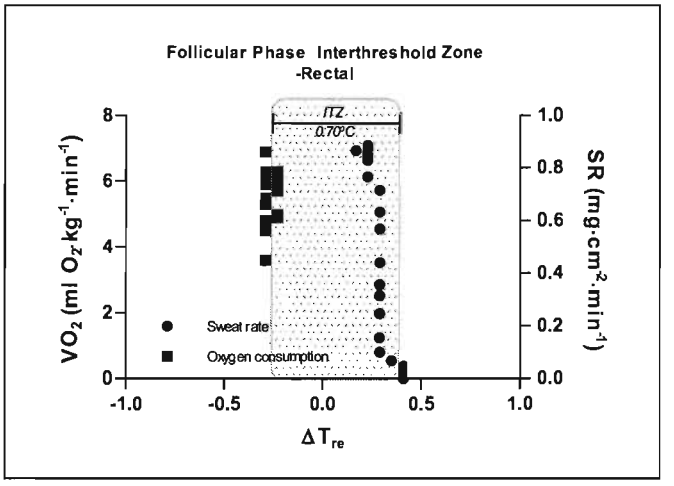


Subject *MK*

Fig 37. and **Fig 38.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



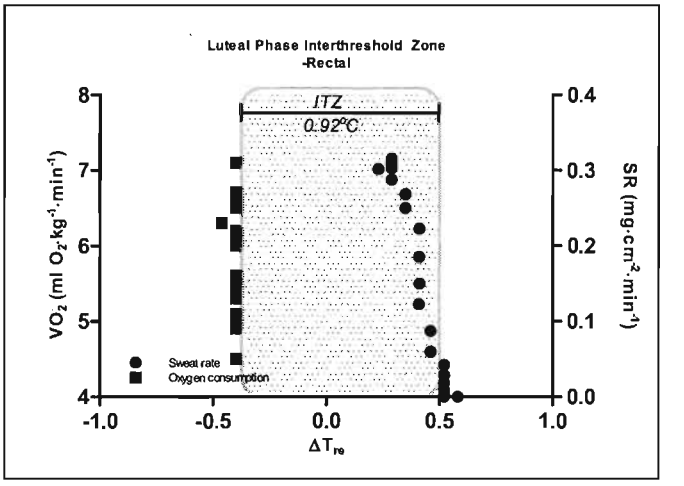
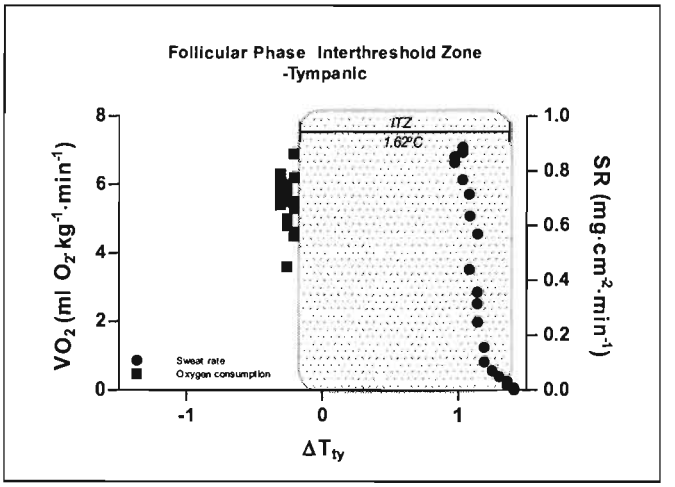
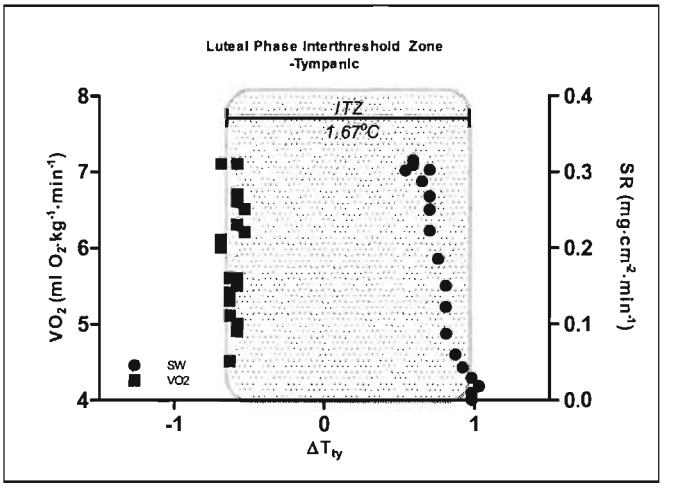
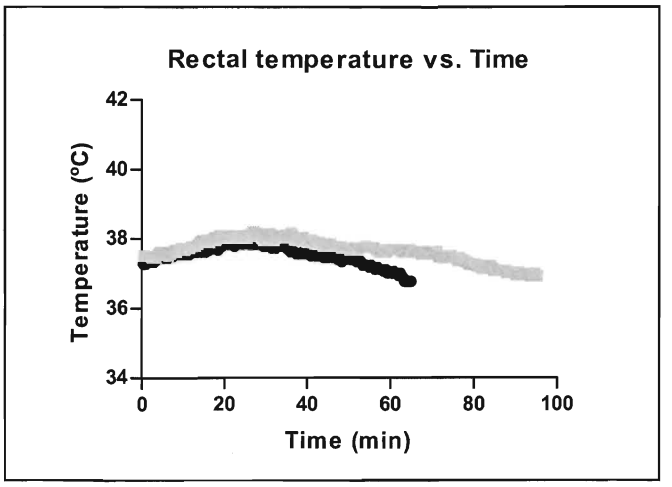


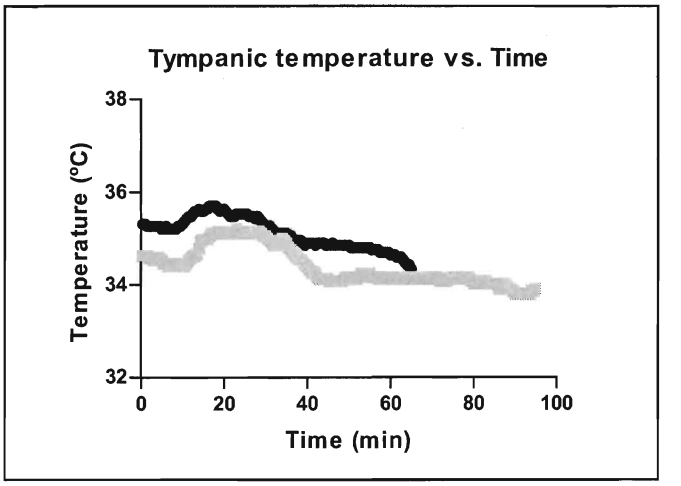
Fig 39. and **Fig 40.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 41. and **Fig 42.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







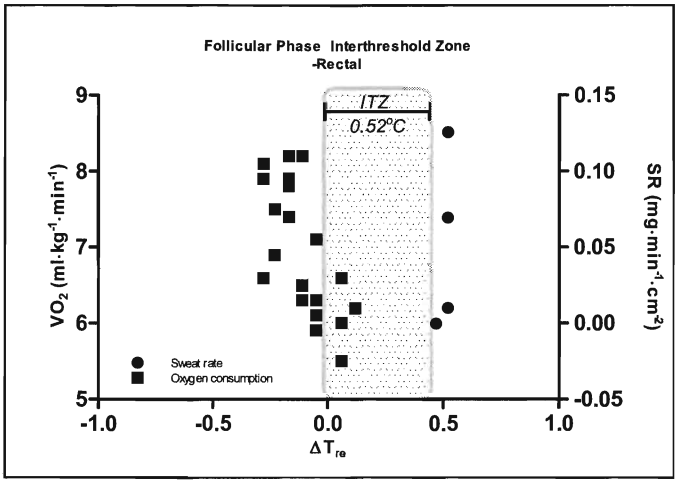


Subject *ND*

Fig 43. and **Fig 44.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



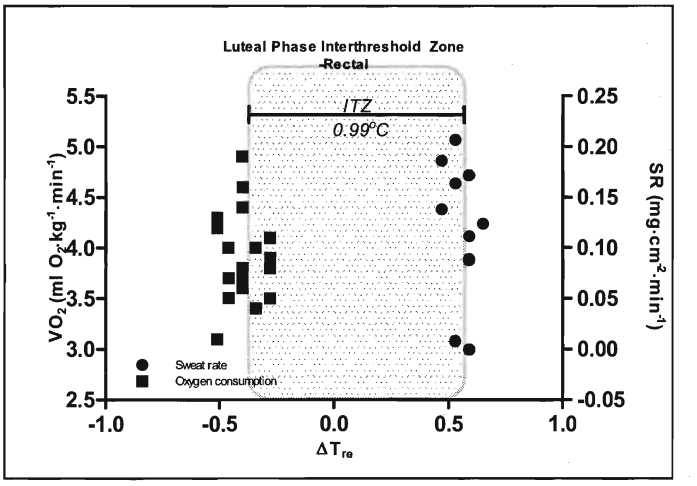
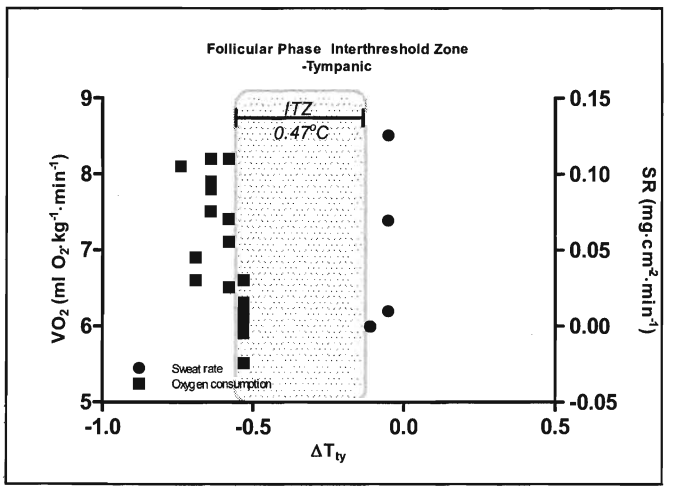
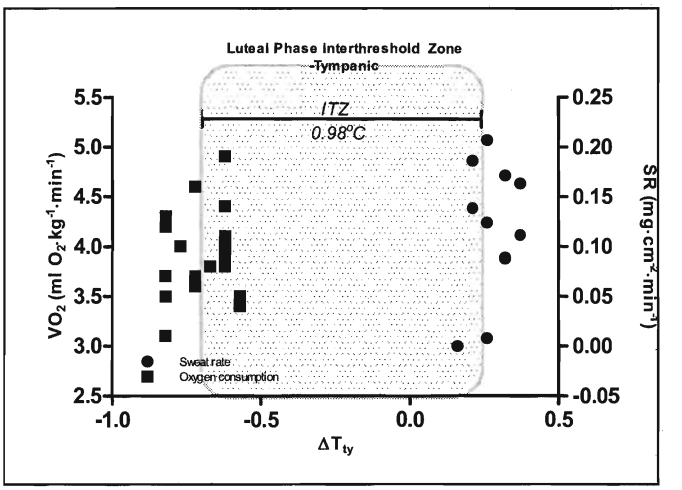
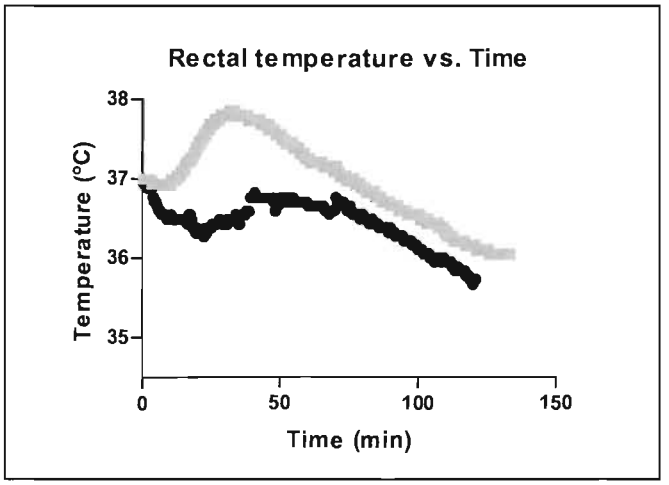


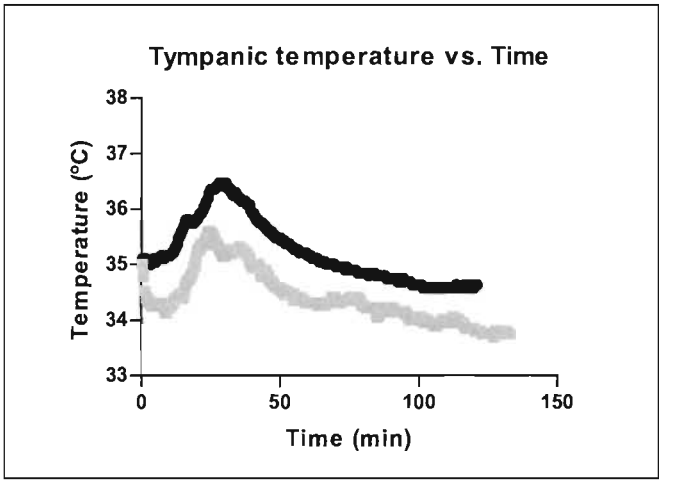
Fig 45. and **Fig 46.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 47. and **Fig 48.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







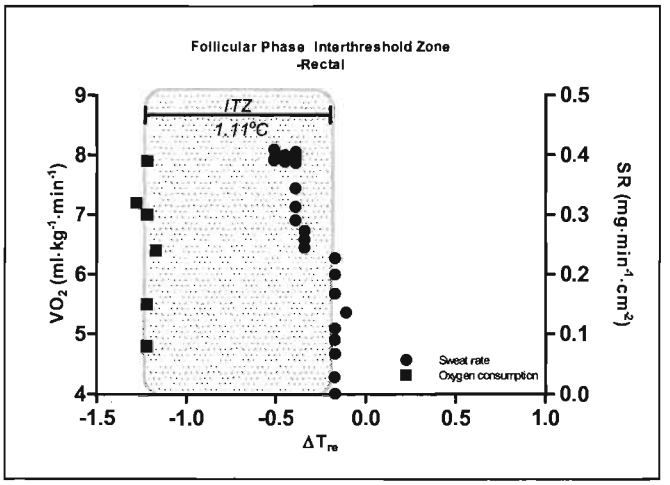


Subject *RC*

Fig 49. and **Fig 50.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



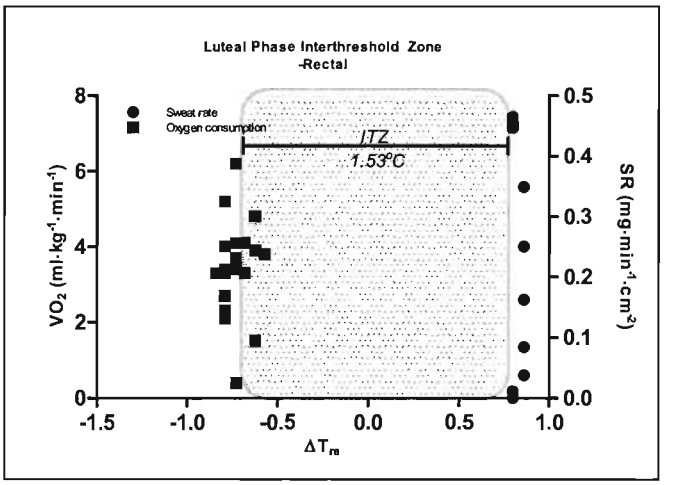
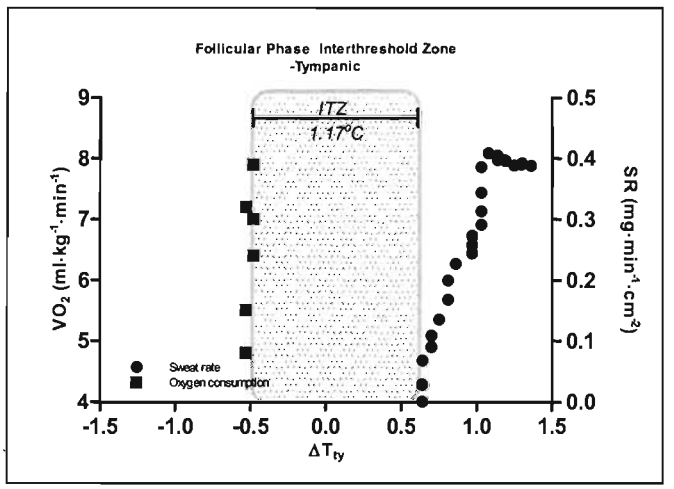
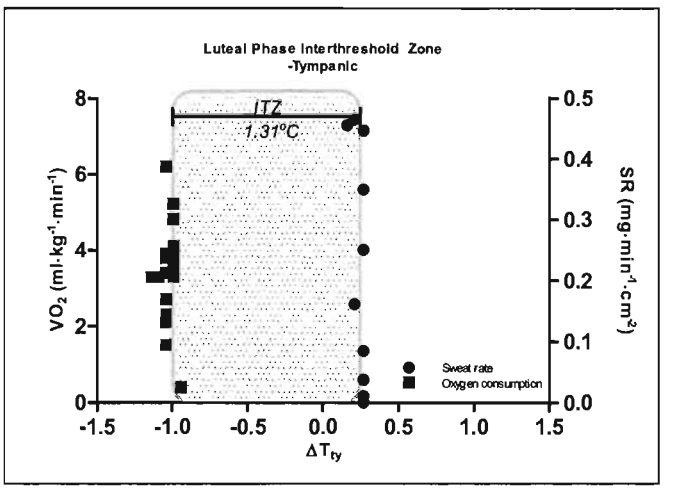
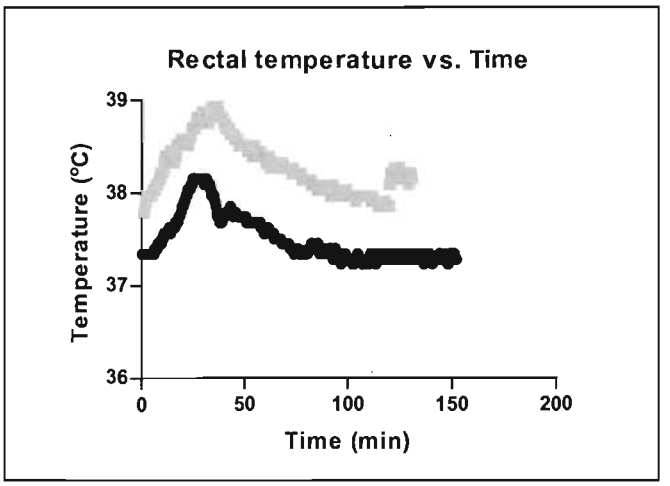


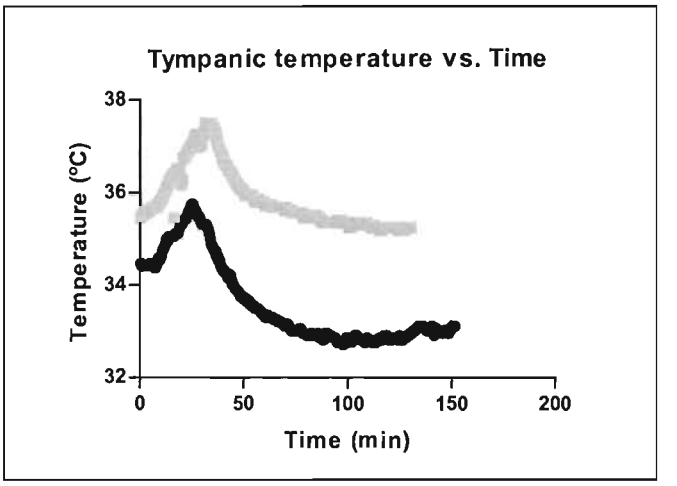
Fig 51. and **Fig 52.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 53. and **Fig 54.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







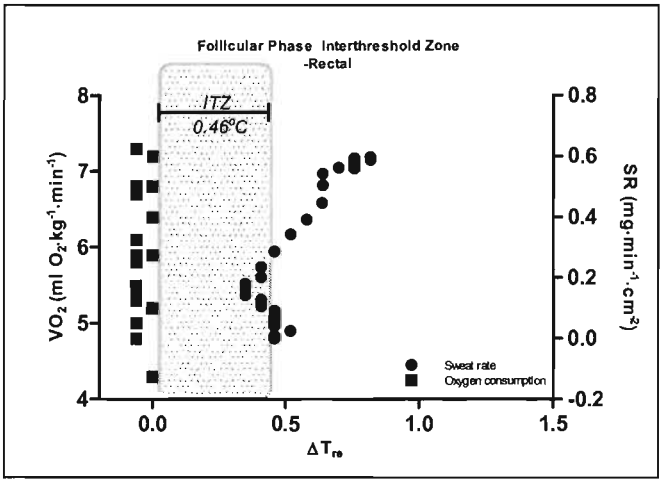


Subject *RK*

Fig 55. and **Fig 56.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



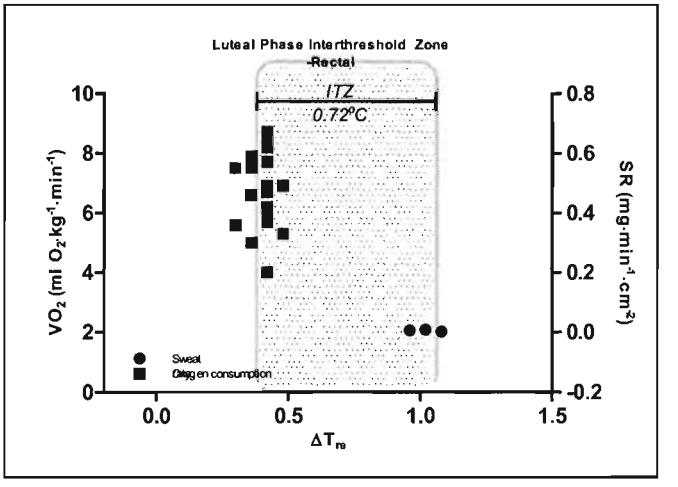
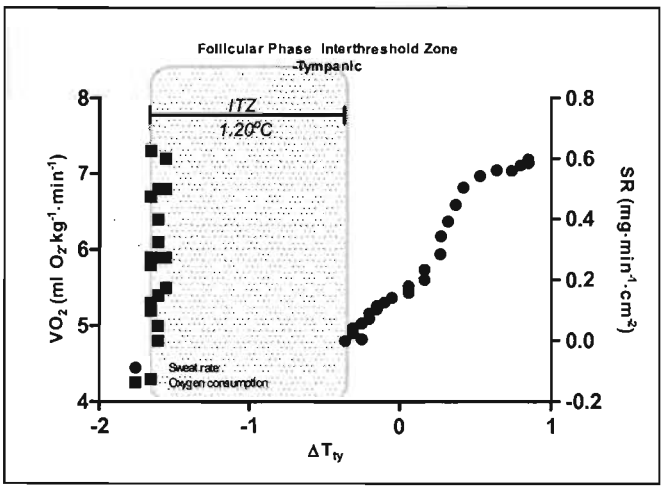
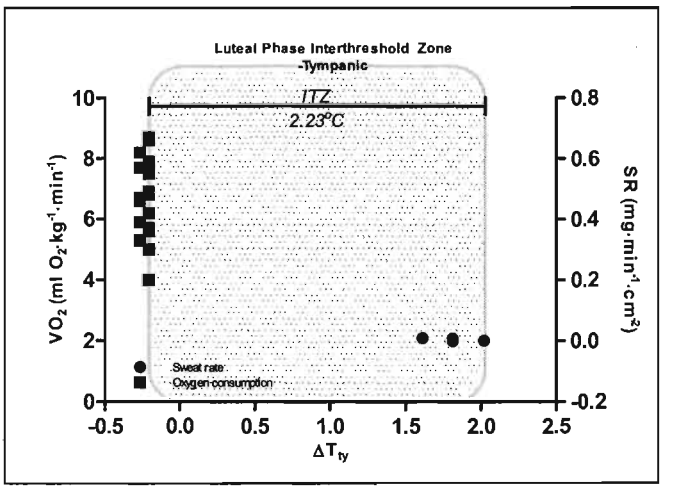
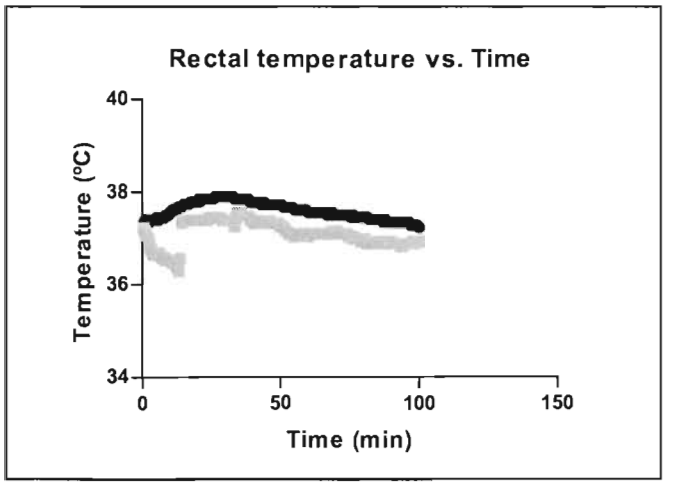


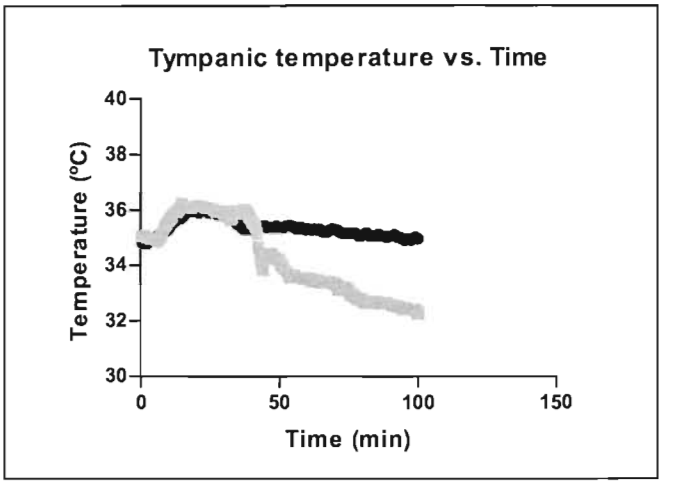
Fig 57. and **Fig 58.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 59. and **Fig 60.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.







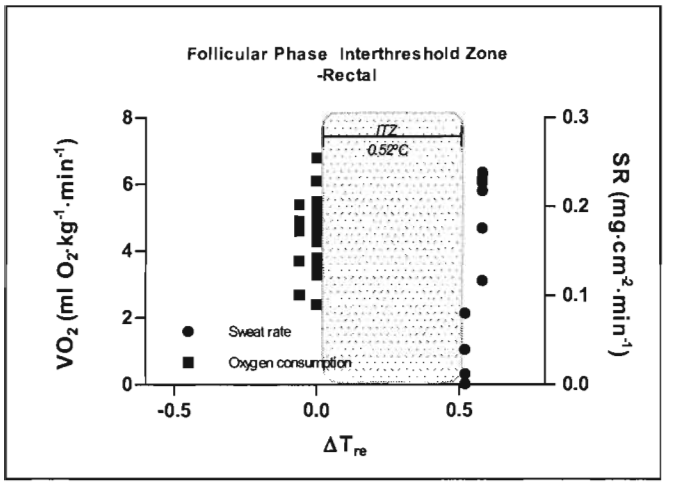


Subject *AL*

Fig 61. and **Fig 62.** Changes in rectal and tympanic temperatures.

● Follicular phase

■ Luteal phase



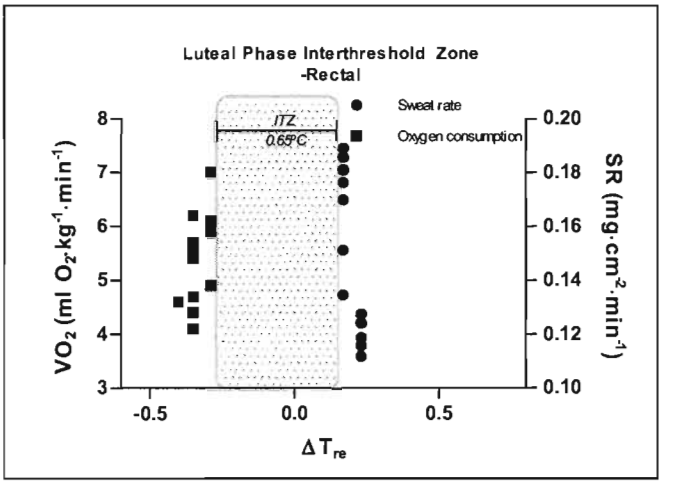


Fig 63. and **Fig 64.** *(above)* Comparison of rectal temperature interthreshold zones between follicular and luteal phases.

Fig 65. and **Fig 66.** *(below)* Comparison of tympanic temperature interthreshold zones between follicular and luteal phases.